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# Effect of Soil Toxicity on the Growth and Development of Crimson Clover (*Trifolium incarnatum* L.) and Blue Alfalfa (*Medicago sativa* L.)



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## Abstract

A vegetation trial was conducted to test, establish and compare the abilities of crimson clover (*Trifolium incarnatum* L.) and blue alfalfa (*Medicago sativa* L.) to grow, sequester arsenic and improve microbial soil status in soils with deteriorated water and physical properties and with naturally and technogenically reached toxic levels of arsenic. The experiment is based on cultivation of two types of legume crops under the same controlled climatic conditions, but in different soil environments. Contaminated Regosols and control uncontaminated Rhodic Cambisols, Chromic-Calcaric Cambisols and Fluvisols, collected from four geographical points, distributed (4 kg.) in a total of 24 pots/samples and sown with the two investigated crops (2x12) were included in the study. Crop growth and development were monitored during the vegetative and reproductive phenological phases, taking timely biometric measurements. According to the observations made and the results obtained, the crimson clover (*Trifolium incarnatum* L.) is suitable for remediation and improvement of the agrochemical, biological, physical and physicochemical properties of arsenic-contaminated soils - Regosols, by forming a dense root system with a relative abundance of tubers of symbiotic nitrogen-fixing microorganisms in it, in conditions of Interventional contents of arsenic, significant compaction and deteriorated water permeability of the soils. Contamination with arsenic (in association with a number of heavy metals) suppresses the germination of both crops - crimson clover and alfalfa, vegetative phenophases, nutrition, growth and development of clover, but not the fertility of the obtained crop of the studied crops. The main factors limiting the fertility of the two crops in the experiment were the low content of soil colloids (humus and clay) and the poorer soil microbiome. The observed better adaptability in feeding alfalfa in contaminated soils allows this crop to be applied in longer-term remediation measures.

**Keywords:** crimson clover, alfalfa, arsenic, phytoremediation, Cambisols, Regosols, Fluvisols

## Introduction

Arsenic and lead contamination of industrial and agricultural lands around the former Kremikovtzi metallurgical plant (near Sofia, Bulgaria) has been studied and documented for decades (Boyajiev et al., 1985; Tchuldjian et al., 1991; Faitondjiev et al. al., 2000; Atanassova, Doichinova, 2003; Shulin et al., 2004, etc.). According to recent soil-genetic studies, which are

unique for the territory of the combine, the nature of the soil-forming rocks, supplemented by long-term human and technogenic influence, plays a role in the exceeded values of a number of metals and metalloids (Stoykova, 2021).

The area of the former MP "Kremikovtsi" is located on a flat-hilly terrain within the foothills of the Sofia valley. The soil-forming proluvial and colluvial rocks contain redeposited material and fragments of a colorful palette of rock varieties, that make up the adjacent mountain slopes - carbonate rocks, argillites, siltstones, lydrites mixed with ore from the Buhov uranium deposit, fragments of rock formations of the very rare potassium-alkaline magmatic series of the Buhovo-Seslavtsi pluton (mainly syenites) and rock material from the Kremikovtsi barite-iron ore deposit (Dyulgerov, 2005; Angelov et al., 2008, Kalaydzhev, 1993). These natural factors presuppose relatively higher background values for a number of chemical elements - heavy metals and metalloids (including As) and lead to the need for sustainable management of soils and lands.

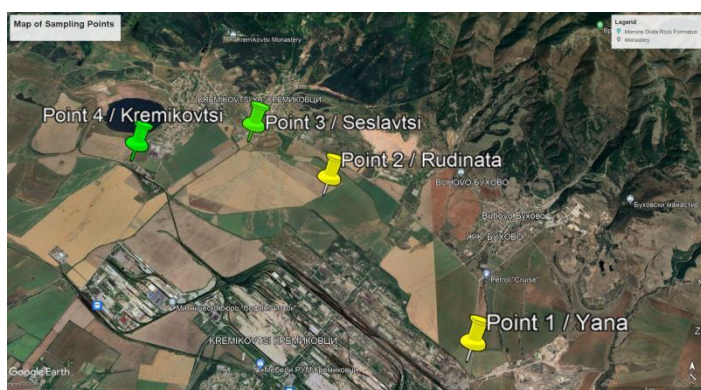
Soil Science has postulated the ability of legumes, thanks to their symbiotic root-dwelling nitrogen-fixing bacterial species, to increase soil fertility by increasing soil nitrogen, and in addition, there is evidence of arsenic-tolerant and even arsenic-transforming nitrogen-fixing bacterial species (Reichman, 2007, Laha et al. 2021). The cited studies allow us to assume that species and strains with similar properties could also be present in the particular region we studied, especially judging by our data, according to which the behavior of As and its related metalloids Se, Te and Sb is the same. Downward removal from the profile and concentration below 200 cm in the BCk horizon is observed only in an uncontaminated control profile, adjacent to which is one of the current control profiles for this study. In all the other 6 technogenically polluted profiles of the cited study, where microbial activity was not detected, these four elements are mainly concentrated in the surface soil horizons, where in places they reach upper limits of 111.01 - 409.34 mg/kg (Stoykova et al., 2022).

In world practice, the crimson clover (*Trifolium incarnatum* L.) has been studied as an arsenic accumulator (Bekuzarova et al., 2018, 2019). For alfalfa (*Medicago sativa* L.), there are a number of studies on an increase in the tissue content of arsenic in contaminated fields (Khattak et al., 1991, Rötting et al., 2013).

Based on the arguments advanced and conclusions drawn, the present study aims to test, establish and compare the abilities of two legume crops (crimson clover and blue alfalfa) to grow, extract arsenic and improve microbial soil status in a vegetation experiment applied on soils with naturally and technogenically reached toxic levels of this pollutant.

## Materials and Methods

To achieve the set aims, based on preliminary publicly available official data (PCSRS of SM, 2021), four sampling points were determined from the most common soil types in the area -

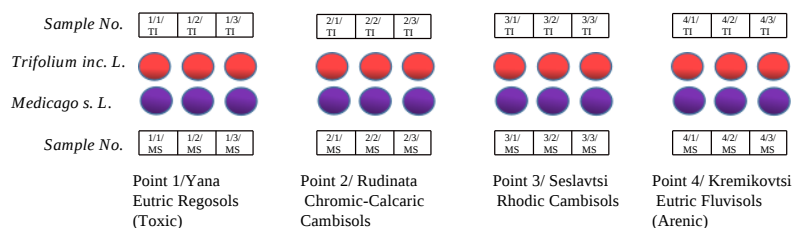


**Figure 1.** Geographical location of the studied soils

Cinnamon forest (Rhodic-Chromic Cambisols), Deluvial soils (Fluvisols) and Regosols (Teoharov et al., 2019; IUSS Working Group WRB., 2022), the latter also classified as Deluvial soils in the used official sources. Accordingly, polluted and non-polluted areas were selected from the soil types (Figure 1). Thus, initially Point 1/Yana and Point 2/ Rudinata were selected as technogenically polluted (respectively,

Regosols and Cambisols), and Point 3/Seslavtsi and Point 4/Kremikovtsi as genetically preserved unpolluted (respectively, Cambisols and Fluvisols).

From each of the four points, in dry winter conditions, on 27/02/2023, 30 kg of soil was collected from horizon A (0-20 cm) of the soil profiles. On the next day, 28/02/2023, the soil was divided into six pots for each of the four study points, each filled with 4 kg of soil and sown in greenhouse conditions with the two crops, each crop in three pots, for statistical reliability of the experiment - a total of 24 pots/samples (8x3 variants) (Figure 2). The crimson clover is the Heuser'S Ostaat variety and the alfalfa is the Gea variety. The seeding rate for both crops is 2 kg/dka (20 mg/100 cm<sup>2</sup>) or 60 mg/ 300 cm<sup>2</sup> (3 dm<sup>2</sup>), which is the approximate area of a pot with a diameter of 20 cm (UC SAREP, 2012; Kirilov, 2016). The initial irrigation rate is 200 ml. every three days (average 70 ml/1 day) for all crops, at an average daily air temperature for the month of March of 7.1°C (-4.8°C - 21.2°C) according to NIMH (National Institute of Meteorology and Hydrology, Bulgaria). Growth and development of cultivated crops were monitored during the vegetative and reproductive phenophases, taking timely biometric measurements (Guertin et al., 2017; Young-Mathews, 2013; GPO, 1960; Kirilov, 2016). As the vegetation progressed, due to a difference in the accumulated biomass and correspondingly greater transpiration, in the mass phase of the second triple leaf (19/04/2023), based on measuring the differences in the weight of the pots for one day, an irrigation rate was determined from 300 ml. every two days (150 ml/1 day) for Regosols and Fluvisols - crops and 500 ml. every two days (250 ml/1 day) for the Cambisols-crops, at an average daily temperature for April of 9°C (-1.7°C - 21.1°C) according to NIMH data. In the phase of inflorescence formation (or bud stage) of the first plant individuals for the entire experiment



**Figure 2.** Schematic of the vegetation experiment

(20/05/2023), all the pots were taken outside, as close as possible to the natural climatic conditions, and the irrigation rates were determined in a timely manner, according to the amount of precipitation, atmospheric temperature,

phytometric indicators and crop transpiration.

On 15/06/2023, the crops grown on Cambisols were collected, and on 22/06/2023 - the crops grown on Regosols and Fluvisols. At this point, the clover in all variants is in an advanced phase of flowering, while this phase in the alfalfa grown on Cambisols is only reached by a few plant individuals. The plant mass was collected by cutting the plants about 2 cm above the root collar, the clover flower was separated from the plants, the resulting biomass was weighed fresh, dried at 60°C to air-dry state, crushed and quartered several times about 2 g of dry matter from each sample were separated and crushed for testing the arsenic content. The total number of samples obtained was 36 - 12 pieces of alfalfa (stems and leaves), 12 pieces of clover (stems and leaves) and 12 pieces of clover flower.

In order to trace the entire vegetation cycle of the plants, three plant individuals were left in each variant pot.

In the laboratories of the Institute of Soil Science, Agrotechnology and Plant Protection "N. Pushkarov" soil samples were tested for:

- pH - potentiometric
- C - Turin method
- mechanical composition - pyrophosphate method



- total carbonates - Scheibler's method
- As - ISO 11466.

Additional samples were taken from the same four sampling points to study the microbiological composition of the soils subject to the vegetation experiment.

## Results and Discussion

The results for the mechanical composition and agrochemical properties of the studied soils are presented in Table 1. According to them, these are light and medium sandy-clay soils with slightly acidic to very slightly alkaline (6.6 - 7.3) pH reaction in water. The highest humus content is Rhodic Cambisols from Point 3 - 4.78 %, followed by Chromic-Calcaric Cambisols from Point 2, Regosols (Toxic) from Point 1 and Fluvisols (from Point 4). The deteriorated water permeability of Regosols and Fluvisols, a result of the low content of the finest fractions and, accordingly, the deteriorated structure of these soils is an important factor that has a significant impact on the development of the studied crops in the current vegetation experience.

**Table 1.** Arsenic content and basic physical and physicochemical properties of the studied soils

| Point No.            | Soil Type                  | pH  | C    | Carbonates | Hygroscopic<br>Moisture | Fine<br>sand | Silt  | Clay | Sum  | As    |
|----------------------|----------------------------|-----|------|------------|-------------------------|--------------|-------|------|------|-------|
|                      |                            | H2O |      |            |                         | 0.25-        | 0.01- |      |      |       |
|                      |                            |     |      |            |                         | 0.05         | 0.005 |      |      |       |
|                      |                            |     | %    |            |                         |              |       |      |      | mg/kg |
| Point 1/Yana         | Eutric Regosols (Toxic)    | 7.3 | 3.25 | 0.98       | 2.3                     | 33.4         | 17.2  | 3.4  | 22.6 | 86.8  |
| Point 2/ Rudinata    | Chromic-Calcaric Cambisols | 7.2 | 3.73 | 6.56       | 4.11                    | 35           | 22.9  | 6.7  | 35   | 14.8  |
| Point 3/ Seslavtsi   | Rhodic Cambisols           | 6.6 | 4.78 | -          | 3.76                    | 23.7         | 14.1  | 11.2 | 31.6 | 20.6  |
| Point 4/ Kremikovtsi | Eutric Fluvisols (Arenic)  | 7.3 | 1.98 | 0.63       | 1.56                    | 37           | 12.6  | 4.1  | 17.9 | 7.5   |

According to the results obtained for arsenic content, toxic levels of metalloid were measured in Regosols (Toxic) from Point 1/Yana. The measured value is 86.8 mg/kg, which is practically within the Intervention norm of 90 mg/kg introduced in Ordinance No. 3/ 01.08.2008. In the control profile of Fluvisols from Point 4/Kremikovtsi, the content is minimal - 7 mg/kg, and in the studied Cambisols, contrary to expectations, the arsenic content is slightly higher in the previously designated as control Point 3/Seslavtsi - 20.6 mg/ kg, compared to the previously declared contaminated, Point 2/Rudinata (14.8 mg.kg), where according to official data based on a 2004 study (Shulin et al., 2004), the metalloid content was tens or even hundreds of mg/kg in the particular locality. After analyzing the properties of this particular soil profile (Point 2), its differences stand out - a high content of carbonates - 6.56 %, a high content of fine sand fraction - 35 % and a distinct maximum content of medium dust fraction - 22.9 %, the latter also affecting the maximum value of the physical clay in this soil sample - 35%, compared to the other 3 studied soils. The question of the reasons for this significant deviation from the preliminary data is debatable. The reasons can be different - biological and pedochemical, but most likely all of them are influenced by the



**Figure 3.** Single pot/samples of the eight variants studied. Plants are in mass simple leaf phase and beginning of first triple leaf phase for Cambisols - crops, 03/31/2023.

termination of the activity of the metallurgical plant and the improvement of the qualities of the local microclimate and landscape.

The vegetation experiment, involving the cultivation of two types of legume crops under the same controlled climatic conditions, but in different soil environments, gave many and divergent results, both for the phenological development of the crops and for their difficulties in growing in the individual soil types. The main difference between the two crops is the length of their growing cycle. Alfalfa is a perennial and the specific type of clover is an annual crop, but their phenophases, like leguminous grasses, are identical (Kirilov, 2016; Lloveras et al., 2001; Young-Mathews, 2013). In our experiment, this difference became noticeable at the beginning of the reproductive period (phenophase of inflorescence formation or bud stage), which in alfalfa in all soil types occurred about twenty days later than crimson clover.

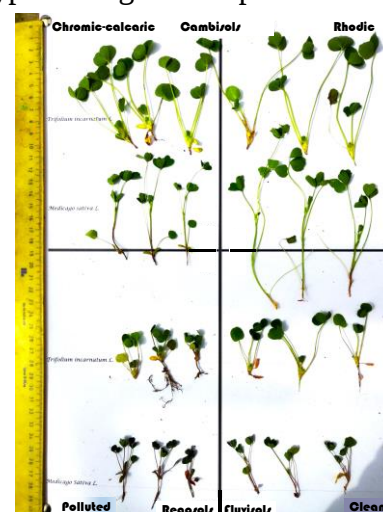
The experiment was carried out on February 28, 2023. The germination phase, manifested by the appearance of cotyledons above the soil surface (GPO, 1960), was delayed by about ten days



**Figure 5.** Top - Crops grown in the third and fourth trifoliate phase. A - Regosols (Toxic), B - Chromic-Calcaric Cambisols, C - Rhodic Cambisols, D - Fluvisols.

Below - Deviations from growth norms - yellowing and closing of clover leaves, 05/05/2023.

structure and agrochemical properties of the two coarser soil types - Regosols and Fluvisols, affected such that the onset of the early phenophases in clover was delayed, but its biometric indicators were not deteriorated (Figure 4, Table 2 - dimensions and yield), in compared to alfalfa. At the end of the experiment, this trend also affects the average yield in fresh and air dry weight, which is greater in clover (Table 2). The more active growth of clover is accompanied (in Regosols, Fluvisols and to a lesser extent in Chromic-Calcaric Cambisols) by yellow-green coloring of the



**Figure 4.** Comparison table, with representative plants from each tested sample/pot, in the third triple leaf phase, with the exception of clover in Regosols and Fluvisols, which is mostly in the second triple leaf phase, 26/04/2023.

for the crops of both crops sown on Regosols (Toxic) - 16 - 18/03/2023, occurring around 7 - 9/03/2023 for

crops of Cambisols and Fluvisols. Germination was suppressed and measured plant density in contaminated Regosols averaged 60 plants/3 sq.m., compared to about 200 in Fluvisols and Chromic-Calcaric Cambisols and over 250 in Rhodic Cambisols (Figure 3). Due to the higher content of humus in Regosols (Toxic) - 3.25 %, the plants manage to gradually synchronize their phases with those of the crops of Fluvisols, which soils in turn have the lowest humus content - 1.98 %.

A general tendency was observed for clover in all soil variants, in the same (Cambisols) or different (Regosols and Fluvisols) phase of development, to have better biometric indicators, compared to those of alfalfa. The deteriorated



leaves, even in the simple leaf phase - a sign of nitrogen deficiency, and in the third and fourth phase trifoliate and with characteristic leaf closure (UC SAREP, 2012; Stanchev et al., 1984) (Figure 5).

As the vegetation progresses and especially after the plants are brought out into the open environment, the deviations related to the delay in their development and difficulty in feeding subside and do not prevent the occurrence of the next phases of all the plants and the completion of the cycle of the few control plants left after the mowing in each sample/pot.

**Table 2. Biometric and phytometric indicators of cultivated crops**

| <i>Trifolium incarnatum</i> L.                | Fresh weight | Air-dry weight | Crop height/ flowering stage/ avg. | Number of blooms |             | <i>Flowers, Trifolium incarnatum</i> L. | Fresh weight | Air-dry weight | Weight ratio Flowers/Green mass | <i>Medicago sativa</i> L. | Fresh weight | Air-dry weight | Crop height/ early bud stage/ avg. |
|-----------------------------------------------|--------------|----------------|------------------------------------|------------------|-------------|-----------------------------------------|--------------|----------------|---------------------------------|---------------------------|--------------|----------------|------------------------------------|
| Sample No.                                    | g            |                | cm                                 | 06.06. 2023      | 08.06. 2023 | Sample No.                              | g            |                | % / avg.                        | Sample No.                | g            |                | cm                                 |
| Point 1/Yana; Eutric Regosols (Toxic)         |              |                |                                    |                  |             |                                         |              |                |                                 |                           |              |                |                                    |
| 1/1/TI                                        | 8.18         | 3.441          | 42                                 | 2                | 6           | 1/1/F                                   | 1.68         | 0.49           | 20.513                          | 1/1/MS                    | 25.4         | 8.8            | 40                                 |
| 1/2/TI                                        | 12.198       | 4.091          | 35                                 | 1                | 3           | 1/2/F                                   | 2.65         | 0.84           | 21.725                          | 1/2/MS                    | 24.2         | 7.9            | 38                                 |
| 1/3/TI                                        | 8.72         | 3.58           | 33                                 | 0                | 1           | 1/3/F                                   | 0.68         | 0.22           | 7.787                           | 1/3/MS                    | 22.6         | 6.8            | 37                                 |
|                                               |              |                |                                    |                  |             |                                         |              |                | 16.675                          |                           |              |                |                                    |
| Point 2/ Rudinata; Chromic-Calcaric Cambisols |              |                |                                    |                  |             |                                         |              |                |                                 |                           |              |                |                                    |
| 2/1/TI                                        | 137.26       | 19.799         | 63                                 | 27               | 33          | 2/1/F                                   | 27.94        | 6.35           | 20.358                          | 2/1/MS                    | 106.1        | 20.1           | 45                                 |
| 2/2/TI                                        | 145.53       | 21.485         | 68                                 | 22               | 36          | 2/2/F                                   | 26.44        | 5.54           | 18.169                          | 2/2/MS                    | 78.0         | 12.4           | 50                                 |
| 2/3/TI                                        | 146.47       | 21.338         | 65                                 | 22               | 32          | 2/3/F                                   | 26.79        | 6.16           | 18.292                          | 2/3/MS                    | 115.8        | 24.5           | 44                                 |
|                                               |              |                |                                    |                  |             |                                         |              |                | 18.940                          |                           |              |                |                                    |
| Point 3/ Seslavtsi; Rhodic Cambisols          |              |                |                                    |                  |             |                                         |              |                |                                 |                           |              |                |                                    |
| 3/1/TI                                        | 199.1        | 28.671         | 69                                 | 12               | 22          | 3/1/F                                   | 29.06        | 5.74           | 14.598                          | 3/1/MS                    | 155.5        | 28.9           | 47                                 |
| 3/2/TI                                        | 210.71       | 31.31          | 74                                 | 10               | 24          | 3/2/F                                   | 26.70        | 4.44           | 12.670                          | 3/2/MS                    | 153.2        | 29.6           | 55                                 |
| 3/3/TI                                        | 272.74       | 43.175         | 71                                 | 13               | 22          | 3/3/F                                   | 34.98        | 7.01           | 12.827                          | 3/3/MS                    | 133.9        | 30.9           | 50                                 |
|                                               |              |                |                                    |                  |             |                                         |              |                | 13.365                          |                           |              |                |                                    |
| Point 4/ Kremikovtsi                          |              |                |                                    |                  |             |                                         |              |                |                                 |                           |              |                |                                    |
| 4/1/TI                                        | 35.328       | 9.476          | 35                                 | 3                | 11          | 4/1/F                                   | 4.28         | 1.65           | 12.124                          | 4/1/MS                    | 38.1         | 14.1           | 30                                 |
| 4/2/TI                                        | 50.964       | 15.647         | 48                                 | 0                | 8           | 4/2/F                                   | 6.70         | 2.61           | 13.147                          | 4/2/MS                    | 39.3         | 13.4           | 27                                 |
| 4/3/TI                                        | 39.283       | 11.641         | 43                                 | 0                | 5           | 4/3/F                                   | 5.15         | 1.89           | 13.102                          | 4/3/MS                    | 31.5         | 8.6            | 28                                 |
|                                               |              |                |                                    |                  |             |                                         |              |                | 12.791                          |                           |              |                |                                    |

The bud stage of clover occurs around 25 - 28/05/2023, respectively earlier for Cambisols and later for Regosols and Fluvisols crops. Flowering occurs on 05/31/2023 for clover sown on Cambisols, on 06/03/2023 on Fluvisols and on 06/05/2023 on contaminated Regosols. In its mass stage, flowering everywhere occurs about two weeks later, when the vegetative trial with the harvest of both crops is completed, and as traced on the control plants left, the phase lasts on average over a month and a half. Alfalfa at the end of the experiment is still in the phase of formation of inflorescences and single (5 pcs.) flowering plants in the Cambisols - the crops (Figure 6).

Depending on the type of soil, the cultivated clover has some characteristic features:

In Cambisols crops, clover growing on carbonate dusty and sandy Chromic-Calcaric Cambisols is distinguished by more abundant flowering with large inflorescences (average, fresh weight 27.1 g from one pot), and low but more branched stems (and fertile branches) and correspondingly more leaf mass (average, fresh weight 143.1 g), compared to that of Rhodic Cambisols, where flowering plant individuals are fewer, plants are the tallest and weakly branched, but the crop as a whole for the experiment is the most abundant in green mass and color, respectively - average, fresh weight 227.5 g. and 30.2 g. (Table 1, Figure 6). It has been estimated



**Figure 6.** Crops grown in clover flowering phase. With a capital letter - alfalfa and clover, with a small letter - clover details. A, a - Regosols; B, b - Chromic-Calcaric Cambisols; C, c - Rhodic Cambisols; D, d - Fluvisols. B, b and C, c - 08/06/2023; A, a and D, d - 15/06/2023.

per pot. Clover grown on Regosols is distinguished by shorter stems, but with more leaf mass and larger flowers than those on Fluvisols - crops. For the Regosols, the weight of the flower was on average 16.7 % of the weight of the green mass, and for the Fluvisols, this percentage was the lowest for the whole experiment - 12.8 %. For the crops of Fluvisols, their numerous but small flowers and the least developed root system are their most distinctive features (Figure 6). The reason may again lie in the lower humus content of Fluvisols. Deteriorated soil structure and water permeability are not the determining factors, since in toxic Regosols these indicators are worse, and plants have more developed reproductive organs (flowers and seeds) and a root system with more lateral and fine branches to the main root, in compared to those of Fluvisols-crops.

The more branched and dense root system of the clover sown on the adjacent Points 1 and 2, respectively Regosols (arsenic-contaminated) and Chromic-Calcaric Cambisols (uncontaminated), was also accompanied by the presence of tubers of symbiotic nitrogen-fixing bacteria at various locations along the main root and branching, which are in equivalent amounts in contaminated and uncontaminated soils. Lumps from base to top and were also observed on the less branched main root of clovers grown on Rhodic Cambisols, but only single ones were found in the root system of Fluvisols crops.

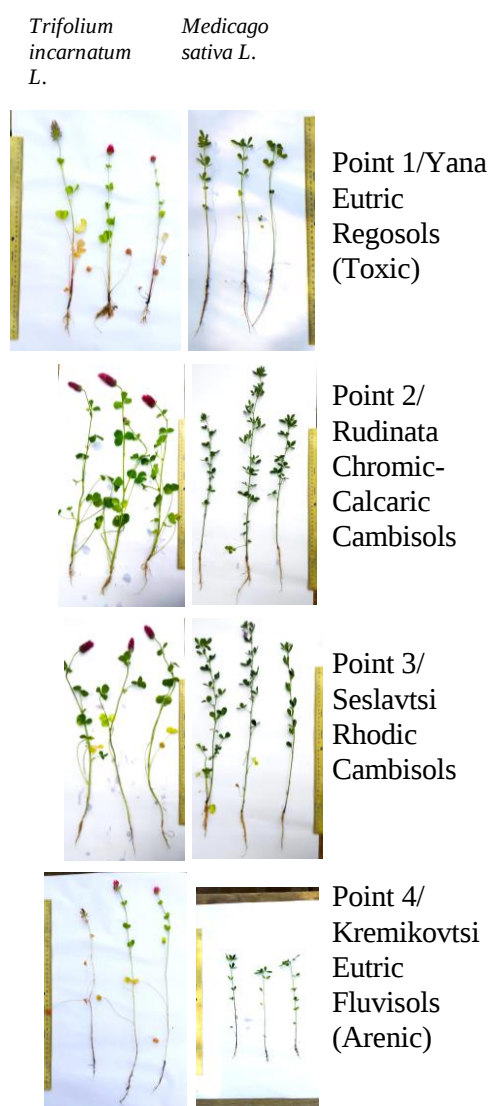
Due to the highlighted observations, it can be concluded that the main factors limiting the fertility of crimson clover in the studied soil samples are the lower content of soil colloids (humus and clay) and the poor soil microbiota, and not the contamination with arsenic (and some heavy metals) (Regosols), the high carbonate content (Chromic-calcaric Cambisols), the high dust content (Chromic-calcaric Cambisols, Regosols) and the high seeding density (Rhodic Cambisols). Arsenic contamination, accompanied by reduced clay content, soil compaction and impaired water

that in clover yield from Chromic-Calcaric Cambisols the flower weight averages 18.9% of the green mass weight, while for Rhodic Cambisols this percentage is 13.4%. It was also observed that the root system of the former is more fragmented and rich in root hairs, compared to the clover of the humus-rich and best-structured Rhodic Cambisols, whose main root is the most powerful, poor in fine root hairs and overtakes the considerably few lateral roots. A reason for these morphological differences could be sought in the degree of germination and seeding density, which are lower in clover on Chromic-Calcaric Cambisols, and there the sparser seeding manages to receive more light and space for development.

The yield of clover was lowest in the toxic Regosols with an average fresh weight of the green mass of 9.7 g and 1.7 g of the flower. For clover grown on Fluvisols, these indicators are 41.9 g of green mass and 5.4 g of color, on average

permeability, has an impact in the initial phases of the vegetative cycle of crimson clover, expressed in poor germination and difficult nitrogen nutrition. The growth of the crop is also suppressed, but this does not affect its fertility and implementation of its main function - symbiotic association with nitrogen-fixing bacteria in its root system.

Alfalfa growth and development, with some deviations, followed the same trends as clover in the studied soil types (Figure 7). The main differences observed between the two crops were the



**Figure 7.** Comparison table with representative plants from each pot/sample (variant) of the two crops at the time of harvest.

lower yield (in three of the points) than the alfalfa (first swath) and the sparsely branched and root system consisting of a deep taproot, rarely branched, along which are single tubers of nitrogen-fixing bacteria. An exception is that in the toxic Regosols the yield of alfalfa was about three times higher, compared to that of clover, and the plants had better biometric indicators than those of the Fluvisols-alfalfa crops. However, more tubers of nitrogen-fixing bacteria were found everywhere in the clover root system than in the alfalfa crop. The development of alfalfa in Fluvisols is most difficult, where it is not delayed, but normal development and the achievement of optimal plant sizes are suppressed. This observation corresponds to some extent with the lower fertility of clover in these soils discussed above, originating from the lower humus and clay content and possibly the poorer soil microbiome.

In none of the variants of the study, alfalfa showed signs of nitrogen deficiency. However, it was observed that in the contaminated Regosols, in the initial vegetative phenophases, until the plants were brought outdoors, alfalfa stems and leaves in all three pots/samples were blue-green in color with a violet tint (Figure 4), a sign of deficiency of phosphorus food for plants in these soils (Stanchev et al., 1984). Despite this shortfall, alfalfa generated nearly three times more yield than alfalfa, suggesting a greater adaptability of the crop in the studied soil differences.

Some questions remain debatable and will be addressed in further publications and trials. On the one hand, to compare the extracted amount of arsenic from the two crops. The question of the influence of the two leguminous crops on the microbiological composition of

the specific soil differences, including the presence and development of arsenic-tolerant species of soil *Rhizobium*, which has been researched to contain symbiotic species of lentils and soybeans, which by different mechanisms, they tolerate and transform arsenic compounds in the soil and, despite them, support the development of host plants (Reichman, 2007, Laha et al., 2021). The probable presence and isolation of arsenic-transforming bacteria in contaminated soils and their interaction with the studied legumes and their symbiotic rhizobia is also a matter of important scientific and applied importance, relevant to the goals of successful and sustainable complex - microbiological and phytoremediation of arsenic-contaminated soils.

## Conclusions

1. According to the vegetative experiment, the crimson clover (*Trifolium incarnatum* L.) is suitable for remediation and improvement of the agrochemical, biological, physical and physicochemical properties of arsenic-contaminated soils - Regosols, by forming a dense root system with a relative abundance of tubers of symbiotic nitrogen-fixing microorganisms in it, in conditions of interventional contents of arsenic, significant compaction and deteriorated water permeability of the soils.

2. Arsenic contamination (in association with a number of heavy metals) suppresses the germination of both crops - crimson clover and alfalfa, the vegetative phenophases, nutrition, growth and development of the clover, but not the fertility of the obtained harvest of the studied crops. The main factors limiting the fertility of the two crops in the experiment were the low content of soil colloids (humus and clay) and the poorer soil microbiome.

3. The growth and development of alfalfa in polluted Regosols is easier and gives a higher yield, compared to crimson clover, but the root system of the plants is less branched and with fewer tubers of rhizobia on them. The observed better adaptability in feeding alfalfa in contaminated soils allows this crop to be applied in longer-term remediation measures.

4. The annual vegetation cycle of crimson clover, the ability to adapt to polluted soils and to generate satisfactory yields of green mass and flowers, as well as the prominent and agronomically valuable property of enriching the soil with more symbiotic nitrogen-fixing bacteria and correspondingly increasing soil fertility for short period, are good reasons for this type of annual crop to find wide application, apart from for remediation purposes, also in green fertilization measures in Bulgarian sustainable agriculture.

## Acknowledgment

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# Dynamics of the Chemical Composition of Precipitation and Input of Elements in an Agricultural Area in Southern Bulgaria



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## Abstract

The study of the chemical composition of precipitation is an important factor in determining the input of elements and the total anthropogenic load in agroecosystems. The study presents the results of a 4-year monitoring of the chemical composition of precipitation from the area of the experimental field Tsalapitsa, Plovdiv on Alluvial-meadow soil (Fluvisol). The effects of chemical elements imported with the atmospheric deposition on vulnerable soils in an intensive agricultural practice were evaluated. The data showed that the investigated precipitations are characterized by a slightly acidic to neutral reaction. A significant seasonal and annual variation of the elements in them was observed, with a higher coefficient of variation reported for cations and nitrogen (61-68%). As a result of the conducted research, the following order of change in the concentrations of cations -  $\text{Ca}^{2+} > \text{NH}_4^+ > \text{Mg}^{2+} > \text{K}^+ > \text{Na}^+$  and of anions -  $\text{SO}_4^{2-} > \text{HCO}_3^- > \text{Cl}^- > \text{NO}_3^- \text{N}$  in precipitation was established. The significant correlation found between  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ( $r=0.92; 0.97$ ) and the weak one between  $\text{NH}_4^+$ ,  $\text{NO}_3^- \text{N}$  and  $\text{SO}_4^{2-}$  suggest a buffer factor against precipitation acidity, and anions concentrations did not represent a risk for a direct negative impact on the soil and the crops grown in the area.

**Keywords:** rainfall, cations, anions, dynamics, agroecosystems

## Introduction

Agricultural production is most affected by climatic conditions and their changes. In recent decades, a significant increase in hydrological extreme events in Europe is seen, which have a significant impact on various aspects of socio-economic life and agro-ecosystems. The extreme climate events are difficult to assess due to their high degree of variability and limited duration of observations (Huo et al., 2020). Rainfall in recent years are considered as an important factor for realizing the productive potential of the soil. Moreover they are significant item of revenue in the balance of the elements in the agroecosystems and represents a way to determining critical loads. The problem of the chemical composition of precipitation is significant, as they effect on the entire ecosystem - soil, plants, waters. According to the studies of certain authors the seasonal fluctuations in temperature and intensive precipitations are the reason for significant losses of nitrate (Mitchell, 2011). The chemical composition of precipitations is the result of climatic conditions, natural

processes and human activities and reflects the characteristics and state of atmospheric pollution (Xing et al., 2017; Zhong et al., 2022). Determining the background state of the atmosphere is becoming increasingly difficult as much of the continent is subject to anthropogenic impacts of local, regional or transboundary origin. According from character and the strength of these impacts, changes occur in the physical- chemical composition and properties of atmospheric sediments. Conducted research Gauger et al., (2001) shows that precipitation contain and transfer chemical elements which in most cases have anthropogenic origin and are defined as pollutants with negative impacts on the ecosystems in which they are deposited and accumulated. Furthermore, passing through the soil the precipitations are filtered and dissolved the elements contained there in, where they can have a significant impact on the quality of groundwater. On the other hand, however, in surface waters (rivers, lakes, etc.), a large part of these elements are undesirable as they cause eutrophication. It is also possible that many plant communities are not able to process into biomass the amounts of nitrogen that come with precipitation, which then enters the surface runoff. Studies Yang et al., (2016) found that even if applied agricultural techniques and fertilization rates are at a high level, wheat and maize yields are also significantly affected by the amount of rainfall. That is why, according to researchers, it is necessary a comprehensive assessment of the role of precipitation and its impact on the entire system is needed (Gornall et al., 2010).

The purpose of the study was to assess the dynamics of the content of chemical elements in the precipitation from the area of the Tsalapitsa experimental field for the period 2020-2023.

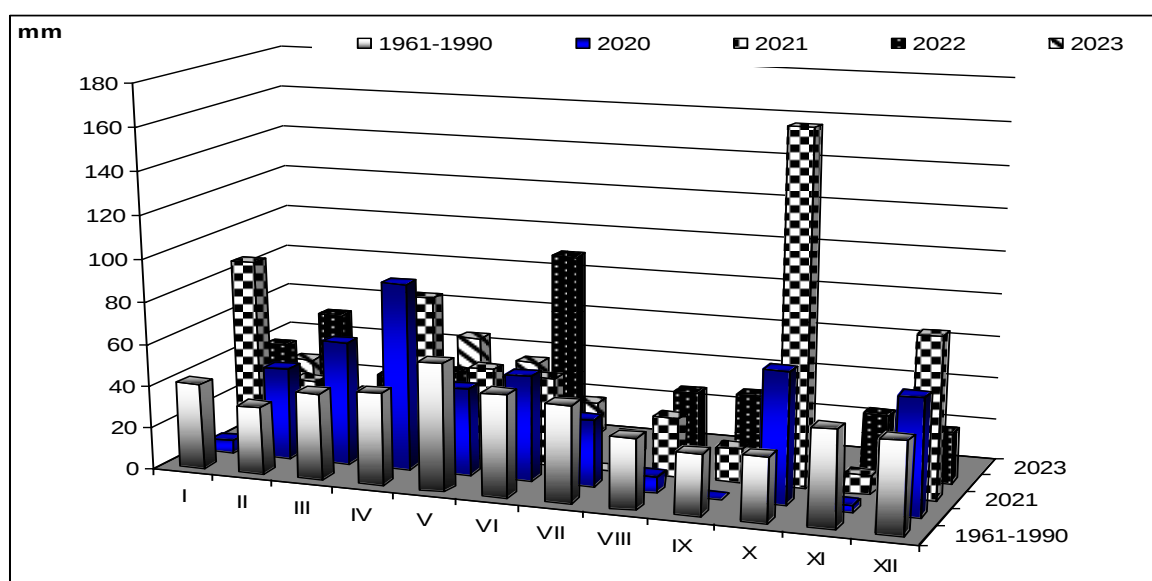
### Materials and Methods

The researched area is located at the experimental field of ISSAPP "N.Pushkarov" in the village of Tsalapitsa, Plovdiv region, South Bulgaria (24°35'E; 42°14'N and 180 m above the sea-level). The chemical composition of precipitation and atmospheric depositions was determined after measuring their volume collected with standard rain gauges (Gyurova and Peev, 1981) after every registered rainfall. Depending on the volume of water and the concentrations of chemical elements their revenue were calculated. Due to the random character in the distribution of precipitation (quantity, frequency and intensity), the chemical composition of individual precipitation was investigated, after which they were averaged and presented with their average monthly values. Regardless of the significant variation in the data, the four-year observations allow obtaining representative average characteristics for the chemical composition of precipitation. The pH values and chemical composition ( $\text{NO}_3^-$ ,  $\text{N}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{HCO}_3^-$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ) of precipitation are determined using the following methods: pH – potentiometrically (Arinushkina, 1970), potassium and sodium were determined by flame photometer, calcium and magnesium – by atomic absorption spectrometry – AAS (Page et al., 1982), nitrogen content, chlorides and sulfates were measured by „Spectroquant Pharo 100”, hydrocarbonate by titration with 0.02nH<sub>2</sub>SO<sub>4</sub> to pH 4.4 (Arinushkina, 1970). The climate in the area is hot with dry summers and mild winters and irregularly rainfall distribution (Levicharska, 1991). The site is characterized by an average annual rainfall of 447 mm for 2020, 601 mm - 2021, 410 mm - 2022 and 150 mm for 2023, which are summarized up to June. The period accepted as a norm 1961-1990 has an amount of precipitation of 492 mm. The average annual air temperature is 12.5-12.8 °C, in July – 23.4, in January it is around zero degrees. In the study area, the soil is Alluvial-meadow (Eutric Fluvisol), (IUSS Working Group WRB, 2015) This type of soil is used for intensive agriculture and is therefore it is suitable for the purposes of the study. The soil profile has a coarse texture, low water holding capacity and fairly high water permeability, which creates conditions for active migration of chemical elements along the profile. The arable horizon has the following mean characteristics: light texture, low clay content (18.6%),

bulk density between 1.54 and 1.66 g.cm<sup>-3</sup>, slightly acidic reaction pH<sub>H2O</sub> (6.0), total nitrogen (0.052 %), humus content - 1.23%, and cation exchange capacity – 22.4 cmol.100 g<sup>-1</sup> (Stoichev, 1997).

## Results and Discussion

The average monthly amounts of precipitation do not affect the values of the investigated parameters that much, but they are extremely important for the development of the cultivated crop and the storage of moisture in the soil. From the obtained data it is established autumn-spring maximum of precipitation, which is generally characteristic for this climatic region. A drought was observed in the summer of 2021 and significantly the amount of precipitation for the month of October - 165 mm (Figure 1).



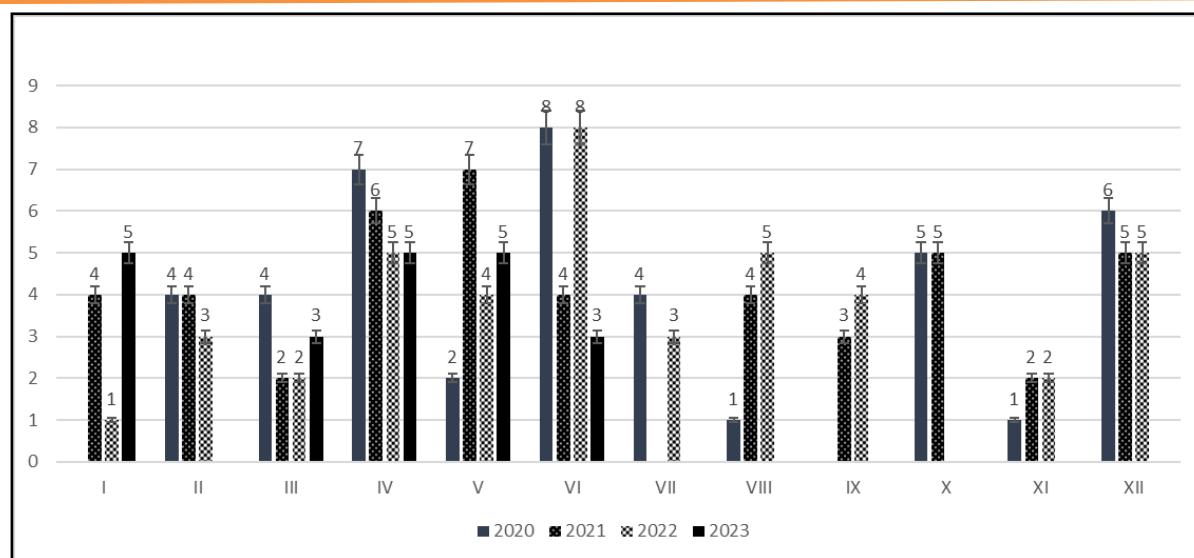
**Figure 1.** Amount of precipitation (mm) for the studied period from the experimental field of Tsalapitsa, Plovdiv and the period accepted as the norm 1961-1990.

In the same year, the largest amount of precipitation was measured - 601 mm, while in 2020 and 2022 they are with similar values, around 410 - 450 mm and approaching the period accepted as the norm. The lowest amount was reported in 2023, at 150 mm, but the measured precipitation was until June. However, compared to other years, the values for the six months for 2023 are about 1.5 - 2 times lower. It was found that the largest amount of precipitation was in the month of April - 88 mm in 2020, while in 2022, the largest volume was measured in the month of June - 94 mm

From the Figure 2, which shows the number of days with precipitation in the respective months for the studied period, it is found that in the spring months - April, May, June, the most days with precipitation were reported.

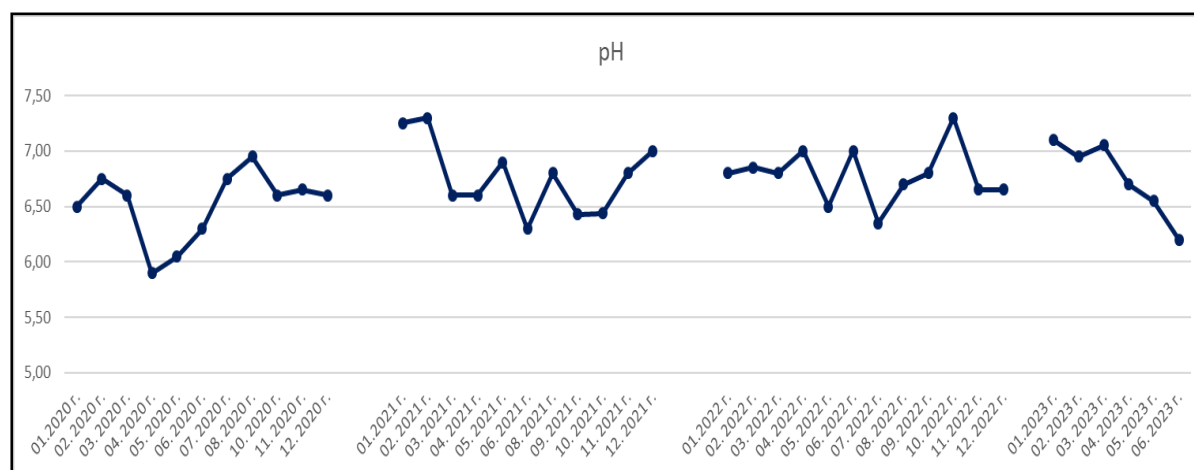
The data show that the precipitation in the study area (Tsalapitsa, Plovdiv region) is characterized by a slightly acidic to close to neutral reaction. During the studied periods, precipitation with an acidic or alkaline reaction was not detected. Some researchers Lara et al., (2010) in their studies reported that the content of calcium ions in the form of CaCO<sub>3</sub> neutralizes the acid components in precipitation. It is possible that the acid potential of precipitation at such a pH is insignificant compared to the neutralizing capacity of soils. It was established that throughout the studied period 2020-2023, the pH values were in the range of 6.5 to 6.8 (Table 1).





**Figure 2.** Number of days with precipitation for the study period 2020-2023

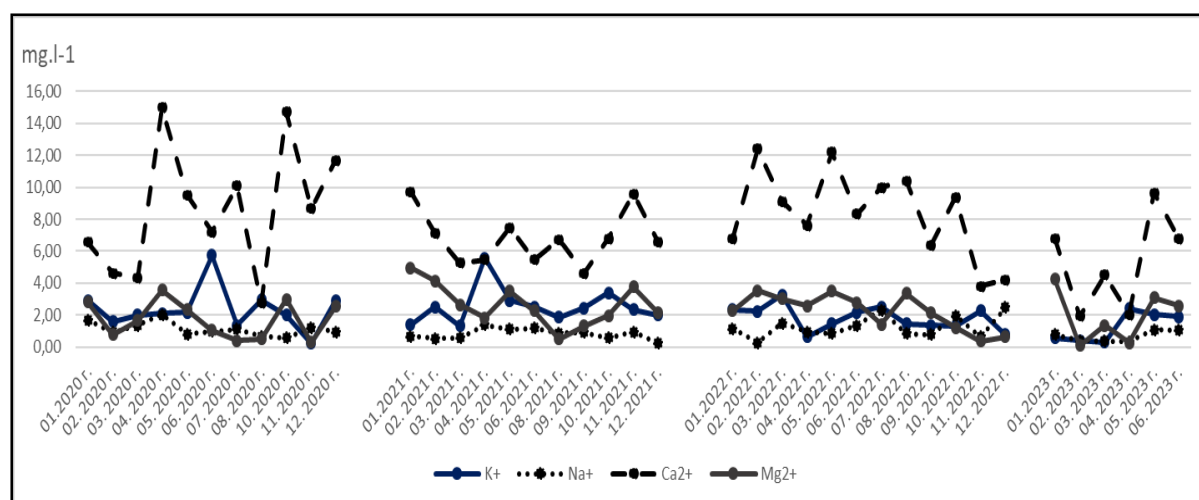
The lowest values reported were in 2020 (Figure 3). It is interesting to note that during the spring months a lower pH values were recorded, while during the summer period the pH values were closer to neutral, i.e. fluctuations in spring are more significant in the slightly acidic zone. This observation is more pronounced in 2020 and 2022. In their research Małeck et al., (2022), have observed the tendency to reduce the acidity of precipitation and improve the quality of its chemical indicators in different parts of Europe which is associated with a transition to more sustainable economies. In a similar study, the authors Keresztesi et al., (2019), found a homogeneous distribution of the analyzed chemical series, which they associated with the implementation of a single European policy in the area of environmental protection.



**Figure 3.** pH values of precipitation in the experimental field of Tsalapitsa, Plovdiv region, during the period (2020-2023).

The results presented in the Table 1 and Figure 4 show that during the studied period 2020-2023, the average content of potassium in precipitation varies from  $(1.81 \pm 0.77)$  to  $(2.56 \pm 1.69)$ , and sodium from 0.81 to 1.25  $\text{mg.l}^{-1}$ , no significant change of the average content of these two ions in precipitation compared to previous periods of the studied area was found. It is believed that their

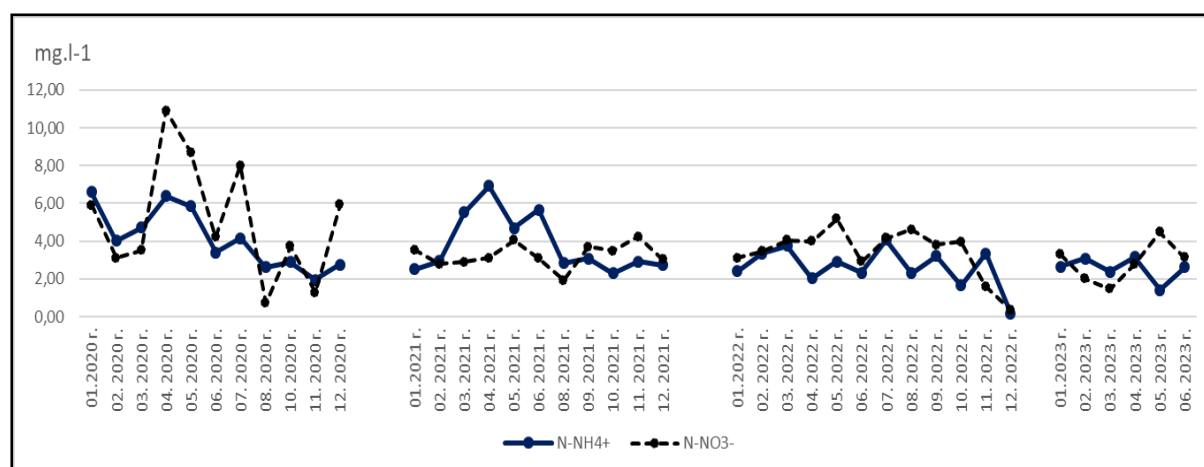
presence is mainly connected with soil dust and that values of about 0.5-0.6 to 3.0 - 3.5 mg.l<sup>-1</sup> are considered background.



**Figure 4.** Dynamics of potassium, sodium, calcium and magnesium in precipitation in the experimental field of Tsalapitsa, Plovdiv region, during the period (2020-2023).

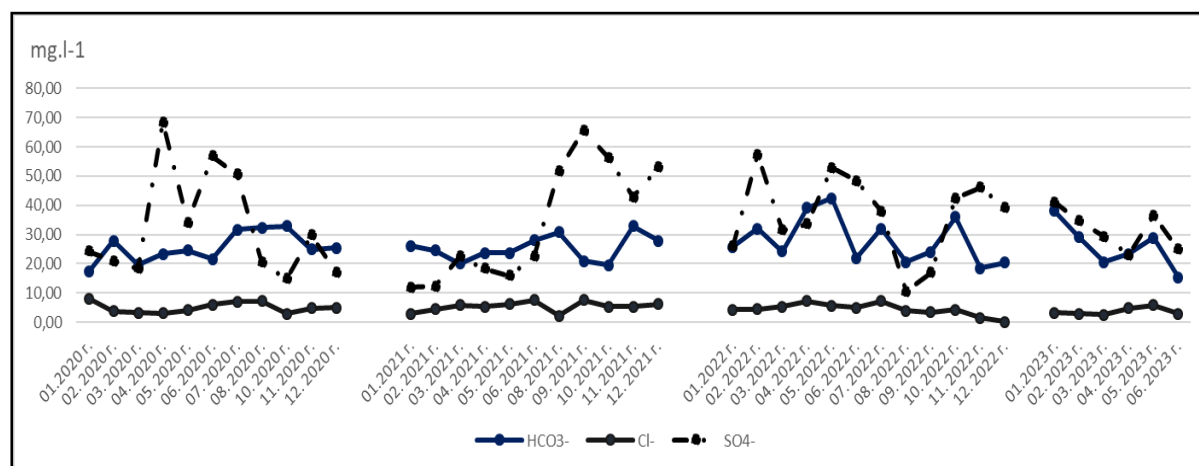
The data show that the content of divalent cations in precipitation is higher in the spring and summer months of 2020, where calcium values reach 15 mg.l<sup>-1</sup>, (average  $8.65 \pm 4.06$ ), and the lowest in 2023 of 1.93 mg.l<sup>-1</sup>, (average  $5.61 \pm 2.93$ ). It is possible that greater dusting is the reason for their concentration in the precipitation. The content of magnesium is about 2.5-3 times lower compared to that of calcium (1.72 - 2.64 mg.l<sup>-1</sup>). However, it has been observed greater variation in its values was observed (Table 1).

The concentration of ammonia nitrogen in 2020, ranges from 1,95 to 6,60 mg.l<sup>-1</sup>, while the reported amount of nitrate nitrogen is in the range from 0.73 to 10.90 mg.l<sup>-1</sup>. The nitrogen content was found to be highest in the spring of the same year, a situation similar to that of calcium and magnesium. As it is shown on Figure 5 ammonia nitrogen concentration in 2021 varies from 2,29 to 6,92 (average  $3.83 \pm 1.58$ ), while for nitrate nitrogen the mean concentrations were  $3.26 \pm 0.64$ . The lowest measured average values of nitrogen were in 2023.



**Figure 5.** Dynamics of nitrogen content in precipitation in the experimental field of Tsalapitsa, Plovdiv region, during the period (2020-2023).

The results for the content of hydrocarbonates and chlorine in the precipitation show insignificant differences compared to earlier study periods in the area of Tsalapitsa. The concentrations of hydrocarbonates in precipitation for the studied period ranged from 25.37 to 28.09 mg.l<sup>-1</sup> with a low coefficient of variation (Table 1). The average values for chlorine are from 4.16 to 5.29 mg.l<sup>-1</sup>, as the chlorine content is 5-6 times lower compared to that of hydrocarbonates ( Figure 6).



**Figure 6.** Dynamics of hydrocarbonates, chlorides and sulfates content in precipitation in the experimental field of Tsalapitsa, Plovdiv region, during the period (2020-2023).

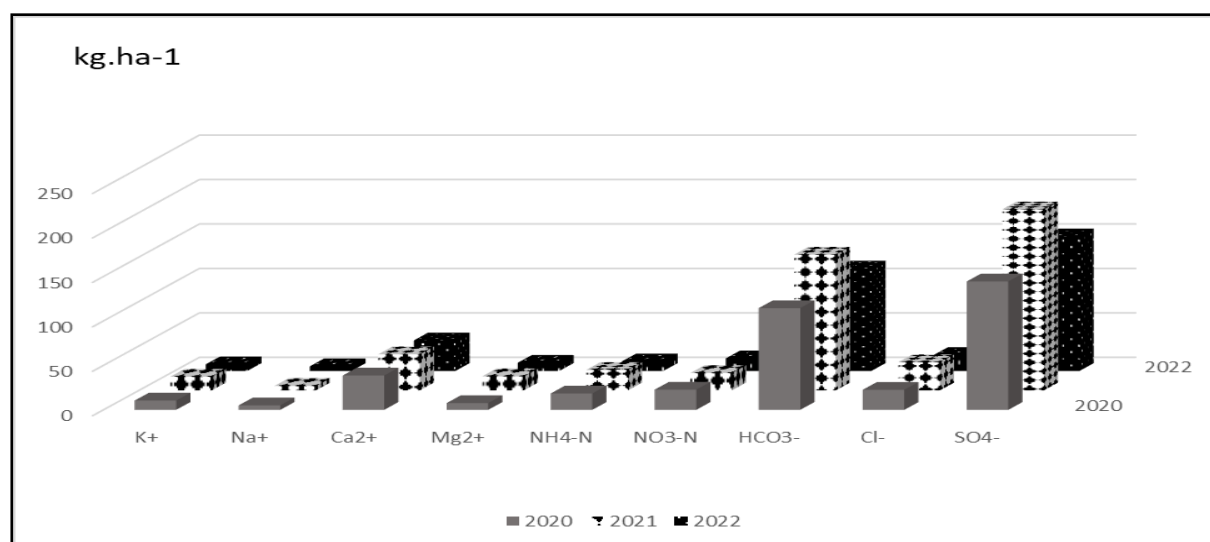
The chemical composition of precipitation shows significant seasonal and yearly trends. The table shows that the dominant ions are Ca<sup>2+</sup>, NH<sub>4</sub><sup>+</sup>, SO<sub>4</sub><sup>2-</sup> and HCO<sub>3</sub><sup>-</sup>. In a similar study, Hontoria, et al., 2003 found that the predominant cations were those of calcium and that they were the main neutralizing agent of nitrates and sulfates, the authors associate this with the stronger influence of the soil in the composition of the precipitation, in contrast to that of the marine influence.

The established significant correlation between Ca<sup>2+</sup> and Mg<sup>2+</sup> (r=0.92 - 0.97) and the weak one between NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> (r=0.15 - 0.22), as well as between NH<sub>4</sub><sup>+</sup> and SO<sub>4</sub><sup>2-</sup>, (r=0.19) suggest a buffer factor against precipitation acidity.

To assess the impact of atmospheric deposition upon the soil it is important to determine the total amount of the ions which included in the annual precipitation. The revenue input of the chemical elements in the precipitation is calculated on the basis an average chemical composition and the total annual rainfall (410 - 600 mm) in the region of Tsalapitsa. From the obtained data, it is established that the input of calcium is from 34.3 to 40.6 kg.ha<sup>-1</sup>, of magnesium 7.6 - 15.2 kg.ha<sup>-1</sup>, with ammonia nitrogen it is within the limits of 10.7 - 20.3 kg.ha<sup>-1</sup>, and with nitrate nitrogen 14.1 - 22.7 kg.ha<sup>-1</sup> (Figure 7). According to Małeck et al., (2022) there has been an increasing trend of air quality improvement in recent years. This trend is also observed in the measured ion concentrations and reduced wet deposition loads of the containing acid-forming compounds. The neutralizing capacity of rainwater is increased by the NH<sub>4</sub><sup>+</sup> it contains, indicating that some of the neutralization takes place in the lower atmosphere. It was found that the average annual input of nitrogen with precipitation for the Tsalapitsa region in previous studies over a 30-year period is about 20 - 23 kg.ha<sup>-1</sup>.

**Table 1.** Chemical composition of precipitation for the period 2020-2023 from the area of the Tsalapitsa.

| Chemical elements, mg.l <sup>-1</sup> |          |                |                 |                  |                  |                    |                    |                               |                 |                               |
|---------------------------------------|----------|----------------|-----------------|------------------|------------------|--------------------|--------------------|-------------------------------|-----------------|-------------------------------|
| 2020                                  | pH       | K <sup>+</sup> | Na <sup>+</sup> | Ca <sup>2+</sup> | Mg <sup>2+</sup> | NH <sub>4</sub> -N | NO <sub>3</sub> -N | HCO <sub>3</sub> <sup>-</sup> | Cl <sup>-</sup> | SO <sub>4</sub> <sup>2-</sup> |
| min                                   | 5,9      | 0,24           | 0,58            | 2,74             | 0,29             | 1,95               | 0,73               | 17,26                         | 2,9             | 14,8                          |
| max                                   | 6,95     | 5,73           | 2,02            | 15               | 3,55             | 6,6                | 10,9               | 32,87                         | 8,1             | 68,4                          |
| mean±                                 |          | 2,36±          | 1,12±           | 8,65±            | 1,72±            | 4,12±              | 5,09±              | 25,61±                        | 5,05±           | 32,31±                        |
| Stdv                                  | 6,5±0,31 | 1,37           | 0,43            | 4,06             | 1,17             | 1,59               | 3,15               | 5,17                          | 1,83            | 18,21                         |
| cv                                    | 5        | 58             | 39              | 47               | 68               | 38                 | 61                 | 20                            | 36              | 56                            |
| 2021                                  |          |                |                 |                  |                  |                    |                    |                               |                 |                               |
| min                                   | 6,3      | 1,4            | 0,24            | 4,6              | 0,5              | 2,29               | 1,92               | 19,52                         | 2,28            | 12                            |
| max                                   | 7,3      | 5,57           | 1,4             | 9,73             | 4,95             | 6,92               | 4,25               | 30,87                         | 7,6             | 65,66                         |
| mean±                                 | 6,8±     | 2,56±          | 0,82±           | 6,8±             | 2,64±            | 3,83±              | 3,26±              | 25,376±                       | 5,29±           | 33,88±                        |
| Stdv                                  | 0,33     | 1,69           | 0,34            | 1,67             | 1,32             | 1,58               | 0,64               | 4,36                          | 1,68            | 20,11                         |
| cv                                    | 5        | 67             | 41              | 24               | 50               | 41                 | 19                 | 17                            | 32              | 59                            |
| 2022                                  |          |                |                 |                  |                  |                    |                    |                               |                 |                               |
| min                                   | 6,35     | 0,62           | 0,25            | 3,81             | 0,35             | 0,18               | 0,37               | 18,51                         | 0,2             | 10,6                          |
| max                                   | 7,3      | 3,24           | 2,47            | 12,4             | 3,54             | 4,06               | 5,18               | 42,35                         | 7,2             | 57,34                         |
| mean±                                 | 6,8±     | 1,81±          | 1,25±           | 8,38±            | 2,23±            | 2,63±              | 3,44±              | 28,09±                        | 4,4±            | 36,92±                        |
| Stdv                                  | 0,24     | 0,77           | 0,68            | 2,76             | 1,1              | 1,06               | 1,32               | 7,95                          | 2,08            | 14,04                         |
| cv                                    | 3        | 42             | 54              | 33               | 49               | 40                 | 38                 | 28                            | 47              | 38                            |
| 2023                                  |          |                |                 |                  |                  |                    |                    |                               |                 |                               |
| min                                   | 6,2      | 0,31           | 0,33            | 1,93             | 0,1              | 1,41               | 1,48               | 15,42                         | 2,50            | 23                            |
| max                                   | 7,10     | 2,39           | 1,09            | 6,78             | 4,25             | 3,15               | 4,47               | 38,03                         | 5,80            | 41                            |
| mean±                                 | 6,75±    | 1,27±          | 0,67±           | 5,28±            | 2,08±            | 2,55±              | 2,87±              | 25,88±                        | 3,73±           | 31,56±                        |
| Stdv                                  | 0,35     | 0,93           | 0,35            | 3,03             | 1,43             | 0,63               | 1,05               | 7,88                          | 1,31            | 7,20                          |
| cv                                    | 5        | 58             | 53              | 52               | 68               | 25                 | 36                 | 29                            | 35              | 22                            |

**Figure 7.** Input of chemical elements (kg.ha<sup>-1</sup>) with precipitation for the studied period

## Conclusions

The chemical composition of precipitation shows significant seasonal and annual dynamics and significant variation the data for potassium, magnesium and nitrate nitrogen. As a result of the conducted research, the following descending order of the concentrations of the cations in the precipitation was established:  $\text{Ca}^{2+} > \text{NH}_4^+ > \text{Mg}_2^+ > \text{K}^+ > \text{Na}^+$  and the anions:  $\text{SO}_4^{2-} > \text{HCO}_3^- > \text{Cl}^- > \text{NO}_3^-$  N respectively. It was found that among the cations, those of calcium and magnesium prevail, followed by ammonium cations, while among the anions, sulfates are followed by hydrocarbonates, and those of nitrate nitrogen are the lowest. Regarding the pH values and the content of chemical elements for 2020 - 2023, it is found that they do not differ significantly from previous periods studied and their concentration does not represent a risk for a direct negative impact on the soil and the crops grown in the area.

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## Content of Radiocaesium in Imported Fruits Sold on the Bulgarian Market



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### Abstract

Monitoring of radiocaesium content in imported cultivated and forest fruits for the production of foods intended for the Bulgarian market and for export was carried out in the period 2021-2022.

The specific activity of the radionuclide determined in all samples was below the maximum permissible value of 600 Bq/kg, specified in EU regulation 2020/1158, being in the cultivated fruits below the minimum detectable activity (MDA).

In forest fruits, the highest content of radiocaesium was measured in bilberries, followed by cranberries and the wild blackberries.

Relatively higher values of the specific activity of Cs-137 were measured in bilberry samples imported from Ukraine, Belarus and Poland.

**Keywords:** radiocaesium, radioactivity of berries

### Introduction

Accumulation of radionuclides in food and raw materials intended for subsequent processing can increase radioactive dose load in humans. Different radioactive elements accumulate in different organs of human body and, depending on their radiotoxicity, may cause pathological changes of varying degrees.

As a result of the Chernobyl accident in 1986, a significant amount of radioactive waste was released in the environment. Radioactive pollution was spread across the territory of the whole European continent in different degree depending on meteorological conditions and the movement of polluted air masses. Deposition of radioactive caesium was 29 PBq in Russia, 15 PBq in Belarus, 13 PBq in Ukraine, and another 27 PBq in other European countries (De Cort et al., 1998).

Physical and chemical properties of radiocaesium are similar to those of potassium. Due to its good solubility in water, caesium is very well absorbed and distributed relatively evenly in the body (Beňová et al., 2016).

The use of various fruits, especially such as the bilberry, in which accumulation of radioactive elements is found (Yablokov, 2009, Kienzl et al., 1992), both for direct consumption and for the production of various food products, requires regular radioecological monitoring.

The EU Regulation 2020/1158 on the conditions governing imports of food and feed originating in third countries following the accident at the Chernobyl nuclear power station (Commission Implementing Regulation (EU) 2020/1158, 2020) specifies the following maximum permissible values for the content of caesium-137 in food:

- 370 Bq/kg for milk and milk products and for foods for infants and small children;
- 600 Bq/kg for all other products.

Monitoring of radiocaesium content in imported cultivated and wild forest fruits was carried out in the period 2021–2022 to ensure the safety of food products intended for the national market and export.

## Materials and Methods

The plant samples tested in the Laboratory in the period 2021 – 2022 included:

- cultivated fruits (frozen and fresh) – strawberries, sour cherries and raspberries;
- forest fruits (frozen and fresh) – bilberries, cranberries, blackberries, wild raspberries, wild strawberries, black elderberries and blackcurrant.

The samples were imported from Sweden, Poland, Latvia, Romania, Serbia, Bosnia and Herzegovina, Turkey, Belarus and Ukraine. The total number of tested samples was 89.

Specific activity of Cs-134 and Cs-137 was determined gamma-spectrometrically by the full energy peaks of both radionuclides,  $E_\gamma = 604.7$  kV (quantum yield – 97.6%) and  $E_\gamma = 661.6$  kV (quantum yield – 85.1%), respectively. The measurement system is product of CANBERRA and includes a DSA1000 multichannel analyzer, pure germanium detector (30% relative efficiency and 1.3 KeV resolution of Co-60 full energy peak at 1332 kV), and GENNIE2K software. The samples were measured in Marinelli-type container of 450 cm<sup>3</sup> volume.

## Results and Discussion

Results of the study carried out in 2021 and 2022 are summarized in Table 1.

The specific activity of the radioactive isotope cesium-134 in all samples was below the minimum detectable activity (MDA), which was expected due to the relatively short half-life of the radionuclide – 2.06 years. This is an evidence that no other deposition of radioactive caesium occurred as a result of fallout or nuclear accidents after the contamination from the Chernobyl accident.

In all fruits analyzed, the specific activity of cesium-137 was lower than the maximum permissible value of 600 Bq/kg specified in Regulation 2020/1158 of EU. The content of the radioisotope was below the minimum detectable activity (MDA) in all cultivated plants.

Cultivated lands are subjected to various tillage as rotational plowing, discing, harrowing, etc. This leads to a significant decrease in the activity concentration of Cs-137 in the upper 10 cm layer of the soil, where the main exchange processes between the plant root system and soil take place (Paramonova et al., 2017). The availability of cesium-137 to plants in arable soils is mainly influenced by the content of clay minerals and the amount of exchangeable potassium. The weathered edges of illitic and micaceous tactoids fraction are the main adsorbent of radiocaesium in the soil and potassium is the main competitor for the specific positions in the soil absorption complex (Absalom et al., 1999). In soils with high clay content, cesium is rapidly immobilized and its bioavailability to plants decrease.

In berries grown on forest soils, however, an increase in the content of cesium-137 was found, being highest in the bilberry, followed by the wild blackberry and the cranberry.

Compared to agricultural lands, forests are complex ecosystems that include several vegetation layers (trees, shrubs, grasses and other annual plants) and multilayered soil profiles (forest litter, semi-organic and mineral layers) leading to different redistribution of radionuclides (Calmon et al., 2009). In undisturbed soils, radiocesium is mainly concentrated in the upper 0-5 and 5-10 cm layers of the soil (Yordanova et al., 2016; Yordanova et al., 2014), making the radionuclide potentially accessible to the root system of plants. An additional factor is the higher contamination of areas of higher altitudes.

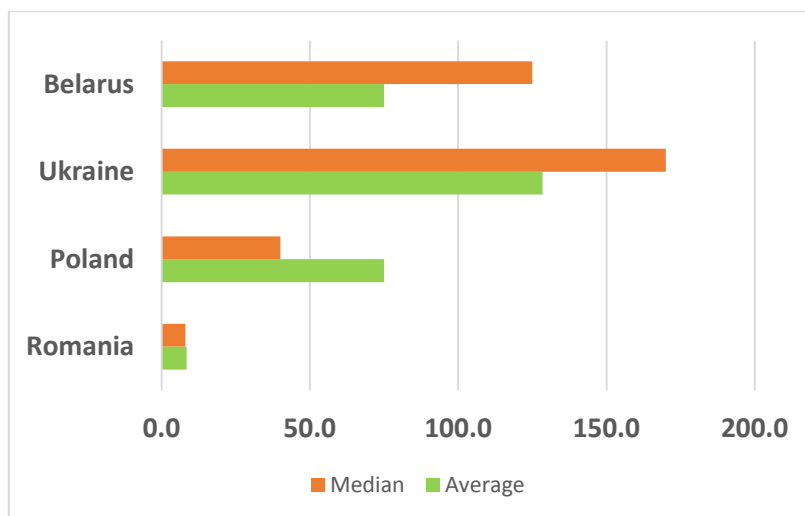
**Table 1.** Content of  $^{137}\text{Cs}$  in imported fruits

| №  | Plant type            | Origin                | Number of samples | $^{137}\text{Cs}$<br>Bq.kg <sup>-1</sup> |        |
|----|-----------------------|-----------------------|-------------------|------------------------------------------|--------|
|    |                       |                       |                   | Min                                      | Max    |
| 1  | Cultivated raspberry  | Serbia                | 3                 | < 1                                      | < 1    |
| 2  | Wild raspberry        | Serbia                | 3                 | < 1                                      | < 1    |
| 3  | Sour cherry           | Serbia                | 7                 | < 1                                      | < 1    |
| 4  | Cultivated strawberry | Turkey                | 6                 | < 1                                      | < 1    |
| 5  | Cultivated strawberry | Ukraine               | 2                 | < 1                                      | < 1    |
| 6  | Wild strawberry       | Montenegro            | 1                 | < 1                                      | < 1    |
| 7  | Wild strawberry       | Serbia                | 1                 | < 1                                      | < 1    |
|    |                       | Bosna and Herzegovina |                   |                                          |        |
| 8  | Wild black strawberry |                       | 1                 | < 1                                      | < 1    |
| 9  | Blackcurrant          | Latvia                | 2                 | < 1                                      | < 1    |
| 10 | Black elderberry      | Romania               | 1                 | < 1                                      | < 1    |
|    |                       | Bosna and Herzegovina |                   |                                          |        |
| 11 | Black elderberry      |                       | 1                 | < 1                                      | < 1    |
| 12 | Wild blackberry       | Romania               | 5                 | < 1                                      | 11±2   |
| 13 | Wild blackberry       | Ukraine               | 2                 | < 1                                      | 30±4   |
| 14 | Wild bilberry         | Sweden                | 4                 | < 1                                      | < 1    |
| 13 | Wild bilberry         | Romania               | 23                | < 1                                      | 15±3   |
| 15 | Wild bilberry         | Poland                | 5                 | 6±1                                      | 180±20 |
| 16 | Wild bilberry         | Ukraine               | 10                | 5±1                                      | 250±30 |
| 17 | Wild bilberry         | Belarus               | 2                 | 40±8                                     | 210±10 |
| 17 | Wild cranberry        | Romania               | 7                 | < 1                                      | 25±4   |
| 18 | Wild cranberry        | Poland                | 1                 | 6±2                                      | 6±2    |
| 19 | Wild cranberry        | Ukraine               | 2                 | 3±1                                      | 5±1    |

Another factor that can lead to the accumulation of radiocesium in the bilberry in particular (*Vaccinum specie.*) is the fact that it belongs to the Ericaceae family, which is characterized by a specific root system growing horizontally within the first few centimeters of the organic layer of the soil, formed by endotrophic mycorrhiza, contributing to the mobilization and absorption of mineral salts from the soil (Calabrese et al. 2018, Ehlken et al., 2002). Higher levels of cesium-137 were found in the cranberry belonging to Ericaceae family too.

Furthermore the higher acidity of forest soils may contribute to the re-mobilization of radiocesium from the soil and increase its availability to the plant.

Average value and median were calculated with the data obtained for the  $^{137}\text{Cs}$  specific activity higher than MDA found in bilberries imported from Romania, Poland and Ukraine. Results are presented in figure 1.



**Figure 1.** Average value and median of the specific activity of radiocesium in bilberries imported from different countries in  $\text{Bq.kg}^{-1}$

The highest content of radiocaesium was found in bilberries from Ukraine, followed Belarus, Poland and Romania, as the median data for Ukraine tended to the higher values of the measured specific activities, while in Poland - to the lower.

The accident at the Chernobyl Nuclear Power Plant resulted in the radioactive contamination of 3.5 million hectares of Ukrainian forests. Soil contamination of the forest massifs with caesium radioisotopes ranged from 37 to 5,200  $\text{kBq.m}^{-2}$ . The most polluted was the region of Polisia, where the area of forests with pollution over 37  $\text{kBq.m}^{-2}$  amounted to 1141.6 thousand hectares (Landin, 2013). In the years after the accident, as a result of the physical decay of radioisotopes, as well under the influence of migration processes, the radiation situation in forest ecosystems significantly changed, as the change depended on a number of factors - homogeneity of the deposition, physical and agrochemical properties of the soils and isotopic composition of the radioactive waste.

The data on the specific activity of radiocaesium in bilberries, collected mainly in the northern regions of Ukraine (Chernihiv, Kyiv, Zhytomyr, Volyn, Khmelnytskyi, Vinnytsia and Zakarpattia regions), cited by Buzynnyi et al. ranged between 69 and 766  $\text{Bq.kg}^{-1}$  (Buzynnyi et al., 2019). Other authors reported even higher values of  $1091 \pm 331.90$   $\text{Bq.kg}^{-1}$  in bilberries from Zhytomyr Region, Ukraine, situated 70 km from the Chernobyl NPP (Davydova et al., 2020). In six batches of bilberries imported from Ukraine intended for the production of jams in Italy, values between  $43 \pm 15\%$  and  $350 \pm 5\%$   $\text{Bq.kg}^{-1}$  were measured (Calabrese et al., 2018).

For Poland, Grabowski et al. reported values of 100  $\text{Bq.kg}^{-1}$  in 1986 and 50  $\text{Bq.kg}^{-1}$  in 1999 for radiocesium content in bilberries collected from different regions of the country, observed regularly on an annual basis (Grabowski et al., 2000).

Belarus was one of the countries which was also heavily affected by the radioactive contamination following the Chernobyl accident. Pollution of forest lands amounted to 23%, decreasing to 16% by 2021. Chernushevich et al. reported data on radiocesium content between 36



and 510 Bq/kg measured in 32 samples of bilberries collected from different regions of Belarus (Chernushevich et al., 2021). In the samples of bilberries imported from Belarus and analyzed in the present study, the content of cesium-137 was between  $40 \pm 8$  and  $210 \pm 10$  Bq/kg.

The results in the present study are comparable to the data from the above-mentioned countries cited by other authors.

Missik et al. cited specific activity of  $535.7 \pm 29.8$  Bq.kg<sup>-1</sup> in bilberries imported from Romania (Missik et al., 2006). These values were higher than the obtained in the present study (from <1 to  $15 \pm 3$ ). The difference was probably both due to the physical decay of radiocesium and the heterogeneity of its deposition.

### Conclusion

The results of the study carried out showed the content of technogenic caesium-137 was below the maximum permissible value of 600 Bq/kg specified in Regulation 2020/1158 of EU in all tested fruits and it can be concluded that they are safe for human consumption both fresh and processed to various food products.

In the cultivated fruits, the measured specific activity of the radioisotope was below the minimum detectable activity.

At the same time, from the data obtained in 2021 and 2022, it can be seen that despite the 37 years period after the Chernobyl accident, well detectable amounts of Cs-137 can still be found in forest fruits such as blackberry, cranberry and bilberry. This requires their radiological monitoring to be carried out on a regular basis, especially on fruits imported from Ukraine, Belarus and Poland.

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# Effects of Cement Dust Emitted by Dangote Cement Factory on Some Chemical Properties of Soils of Tse-Kucha, Benue State, Nigeria



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## Abstract

Some chemical properties of soils were assessed to determine the effects of cement dust emitted by the Dangote Cement Factory on the soils of Tse-kucha community within which the factory is situated. A soil profile pit was dug at Tse-kucha and soil samples were collected from each horizon of the profile pit for laboratory analysis. For comparison, samples were also collected from another profile pit dug at Gaando, a nearby community to Tse-kucha where there are no cement dusts in soils. The samples were analyzed for their chemical properties. Laboratory determinations made include; pH, organic carbon, exchangeable bases; Ca, Mg, Na and K, exchangeable acidity, available phosphorous, total nitrogen, CEC and percentage base saturation. The results obtained from the laboratory analysis of soils from the two study sites were then compared. The result showed that, pH and organic carbon content were significantly higher in soils of the affected areas than those of the unaffected areas due to the continuous deposition of cement dusts on the surface and leaching into deeper horizons. Also exchangeable acidity available phosphorous were slightly higher in soils of the affected areas, the result also showed that, exchangeable potassium, magnesium, total exchangeable bases, and cation exchange capacity were slightly higher in the unaffected soils but there was no significant difference in terms of total nitrogen, exchangeable cation and sodium, and percentage base saturation. Generally, the research showed that there was no significant difference in chemical properties between soils of the two study sites. Thus presently, the cement dust from the factory do not significantly affect the chemical properties of soils of the study area within which the factory is situated as the dust deposits only led to a slight but insignificant change in a few chemical properties but the variation is not significantly enough to cause a serious adverse effect on crop production.

**Key words:** chemical, cement dust, emitted, profile, soil properties, Tse-Kucha

## Introduction

The establishment of any industry is basically and essentially to create wealth for the purpose of development and the provision of social and economic amenities. All these are aimed at the well-being and advancement of mankind. It is in such regard that the Dangote Cement Company was established in Tse-Kucha, Gboko local government area of Benue state. The establishment of

the industry as laudable as it may be has created problems to the area within which it is located (Tse–Kucha, Tse–Amua and Yandev) as a result of cement dust emission. It is on this basis that the research was set up. The research intends to identify the effects of the emitted cement dust from the company on the soils of the community within which it is located and possibly recommend ways of correcting such problems or effects so that the soils can become more useful for agricultural purposes and also seek ways to put a lasting end to the problems.

Cement is a powdery substance made by heating lime and clay, used to make mortar and concrete. The major raw material for cement production is limestone. Cement dust/cement kiln dust is a by-product of Portland cement manufacturing process. It is generated from burning the raw materials in a rotary kiln to produce clinker (Yahya, 2012). Cement factories are factories that are specialized in the manufacture, packaging and marketing of cement. Cement factories were established in Nigeria in response to the increased wave of construction after independence and during the oil boom era of the seventies (Oyedele *et al.*, 1990). It has been shown that cement factories constitute one of the worst polluters in Nigeria today (Akeredolu *et al.*, 1994). Establishment of these plants pre-dates strict industrial zoning regulations and hence their proximity to residential areas is now an issue of concern.

The soil is a thin layer of material on the earth's surface in which plants have their roots. It is made up of many things such as weathered rocks and decayed plant and animal matter. Soil is formed over a long period of time. Soil forms an integral part of the environment. The environment refers to the physical and biological components of the earth along with their chemical interactions that affects an organism. These components also include the soil which is the major study tool in this research.

Industries and factories whether private or government owned engage in production activities for the purpose of making profit. This sometimes endangers human health and the environment which includes the soil that is of great emphasis in this research.

Government in recent times beginning from the federal down to the local government level have been adopting ways of minimizing the effects of industrial pollution. Also, the federal and state governments set up bodies and programmes and annually recruit men into the bodies and programmes to reduce the effects of industrial pollution in the society. More worrisome is the seemingly unrealistic nature and inefficiency of these organizations in the reduction of industrial pollution in spite of huge government expenditure on environmental maintenance like the Benue State Environmental Sanitation Authority (BENSESA) among others and one would observe that, as more strategies are devised by the government to tackle the issue of industrial pollution, so too are the persistent increase of industrial pollution as a result of the increased number of industries in the society. The media on daily basis broadens our knowledge on the increasing cases of industrial pollution within Tse–Kucha community in Gboko area of Benue state within which the Dangote Cement Company is situated. The Dangote Cement Company, whose establishment in Gboko area has created a lot of problems to the host communities (Tse–Kucha, Tse–Amua and Yandev) between 1981 when it was established as Benue Cement Company till date (2014) as a lot of cement dust has been emitted into the environment thereby affecting the socio-economic activities of the host communities. Based on the above, the study is set to assess the effects of the emission of the cement dust from the Dangote Cement Factory on the soils of the host community (Tse–Kucha) and to proffer possible solutions to curb the problems.

Every manufacturing industry that uses raw materials has by-products which may be waste products that can be in solid, liquid or gaseous form. As wastes, they are generally disposed into the

environment of air, water or land where they may constitute serious hazard but when adequate precautions are taken, these wastes will not be hazardous. This study was embarked upon to assess the effects of cement dust emitted by the Dangote cement factory on some chemical properties of soils of Tse-Kucha community in Gboko area of Benue State.

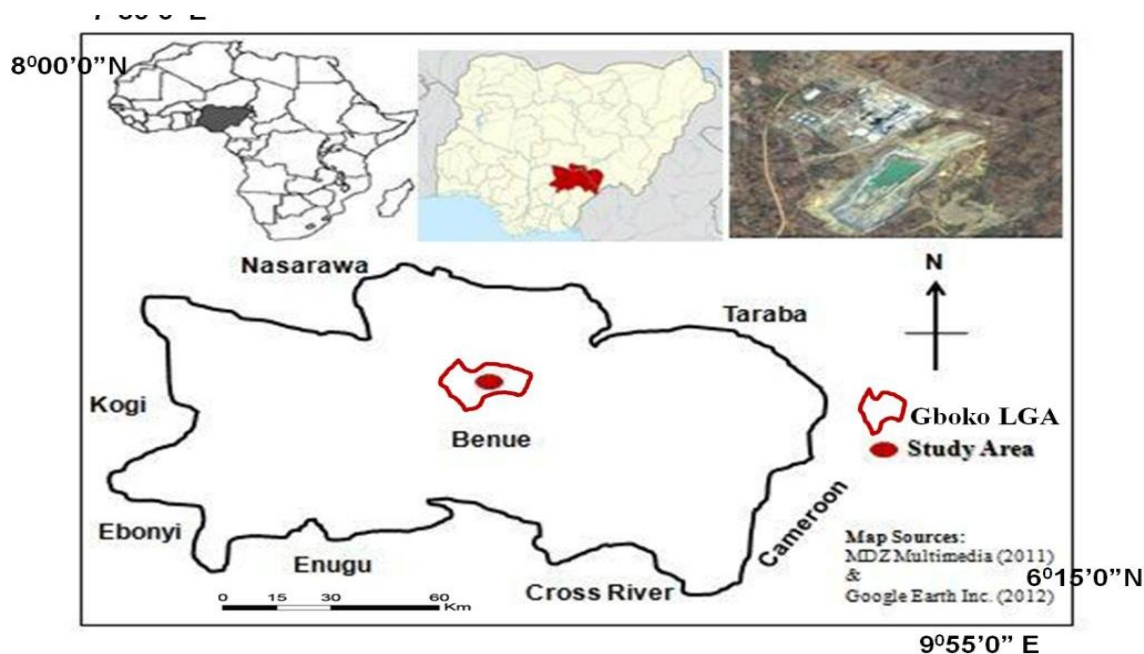
## Materials and Methods

### Study Area

Tse-Kucha is a community in Gboko local government area of Benue state. Tse-kucha lies within latitude  $7^{\circ} 25' 26.4''$  N and longitude  $8^{\circ} 58' 8.4''$  E. The elevation of Tse-Kucha is about 150 m above sea level. Generally, the area has a tropical climate. The climate here is classified as Aw (tropical rainy) by the Koppen-Geiger system. The average annual temperature in Tse-Kucha and Gboko as a whole is  $26.8^{\circ}\text{C}$ . It has a mean annual rainfall of about 1412 mm. The driest month is January with 3 mm. Most precipitation takes place in September with an average of 264 mm. The warmest month of the year is March with an average temperature of  $29.4^{\circ}\text{C}$ . In July, the average temperature is  $25.4^{\circ}\text{C}$ . It is the lowest average temperature of the whole year (NIMET, 2014).

The soils in Tse-Kucha are mainly sandy soils with clay sub-soils and the soils have a characteristic reddish colour. The soils are mainly Ultisols (tropical ferruginous) which vary over space with respect to texture, drainage and gravel content. A typical soil profile in Tse-Kucha is highly weathered with a sandy surface layer overlying a clay mottled sub-soil.

The vegetation of Tse-Kucha is that of the southern guinea savannah and the major type of land use is agriculture, building and few constructions. The occupation of the people of Tse-Kucha is mainly small-scale farming and petty trading.



**Figure 1.** Map of Benue State showing the study area.

### Sample collection

Soil samples were collected from soil profile pits dug around the factory at a range of 0.8 km south of the factory. Basic method of soil profile research was used, one at the affected area (Tse-Kucha) where there has been cement dust deposition on soils for years and the other at Gaando where there are no cement dust deposits on soils to serve as the control area. Soil profiles were



described according to FAO (2006) guidelines while the soils were classified in line with USDA (2010) Soil Taxonomy. The various genetic horizons were then sampled for laboratory analysis. The samples were air-dried, crushed and passed through a 2 mm sieve for laboratory analysis.

### Laboratory analyses

Standard procedures were employed in laboratory analysis. Laboratory determinations made include the following: pH (1:2.5 suspension in water), organic carbon (Walkley and Black, 1934 method), Exchangeable bases: Ca, Mg, Na, and K (extracted with 1N ammonium acetate at pH 7.0), exchangeable acidity (extracted with 1N KCl), available phosphorus (bray 1 method), total nitrogen was determined using the Kjeldahl procedure (Bremner, 1965) and the ECEC was calculated using the summation method.

### Statistical analysis

T-test was the statistical technique used to test the significance of the difference in soil quality between the two study sites.

### Results and Discussions

The chemical properties of soils as indicted in Table 1 shows the affected area (Tse–Kucha community) where there has been cement dust deposition for years, and unaffected area (Gaando Community) where there is no cement dust deposition on soils.

The pH of soils of the affected area had values ranging from 5.8 to 7.7 and was moderately acid to slightly alkaline when compared with the ratings of Chude *et al.* (2011). This is due to the deposition of cement dust on the soil. The soils pH of the unaffected area was strongly acid to slightly acid with values ranging from 5.5 to 6.0. The higher pH values in soils of the affected area were due to the deposition of cement dust on the soil because cement dust contains lime which is known to increase soil pH. The T-test results as presented in table 2 showed that the difference in pH between the two study sites was highly significant with a p-value of 0.001. Organic carbon content was slightly higher in soils of the affected area than those of the unaffected area having values between 0.04 to 0.80 and 0.98 to 1.36 respectively. According to Igomu and Idoga, (2017) the low organic carbon content of these soil units could be attributed to annual bush burning in the area as well as the native vegetation cover and climate. Drainage appeared to have strong influence on the organic carbon content of the soil. This can also be attributed to the accumulation and subsequent deposition of plant residues on the soil surface which are a source of carbon to the soil.

There is only a slight variation in available phosphorus between the two study areas. It is just slightly higher in the affected area than in the unaffected area. One of the factors that influences the availability of the element is soil pH (Brady and Weil, 1999; Landon, 1991). It becomes much more available at pH levels between 6.0 and 8.0 as found in the study area and becomes less available at lower pH levels (> 6.0) as found in the unaffected area, where the element is liable to be fixed by Al, Fe, and Mn (Euroconsult, 1985; Mizota and Van Reeuwijk, 1989). Since the pH of soils of the affected area lies mostly between 6.0 and 7.0, they contain more available phosphorus. There is no significant difference in the total nitrogen content between the two study sites and total nitrogen content was generally low in soils of both the affected and unaffected areas with values ranging from 0.05 to 0.06. Table 2 also shows that the difference in total nitrogen content between the two areas was insignificant with a p-value of 0.

**Table 1. Chemical properties of the study areas (Tse-Kucha and Gaando Communities)**

| Horizon                      | Depth   | pH<br>(H <sub>2</sub> O) | OC<br>(%) | TN<br>(%) | Av.P<br>mg/kg | K    | Na   | Mg   | Ca   | EA   | TEB  | CEC<br>(cmol/kg) | BS<br>(%) |
|------------------------------|---------|--------------------------|-----------|-----------|---------------|------|------|------|------|------|------|------------------|-----------|
| Soils of the affected area   |         |                          |           |           |               |      |      |      |      |      |      |                  |           |
| Ap                           | 0-32    | 5.87                     | 1.2       | 0.058     | 0.34          | 0.28 | 0.32 | 2.6  | 3.1  | 1.1  | 6.3  | 7.4              | 85.1      |
| B                            | 32-58   | 6.42                     | 1.36      | 0.061     | 0.38          | 0.3  | 0.36 | 3.21 | 3.42 | 1    | 7.29 | 8.29             | 87.9      |
| Bt1                          | 58-86   | 6.6                      | 1.04      | 0.056     | 0.32          | 0.27 | 0.31 | 3    | 3.2  | 1.01 | 6.78 | 7.79             | 87        |
| Bt2                          | 86-107  | 7.76                     | 0.98      | 0.057     | 0.3           | 0.24 | 0.3  | 2.8  | 3.01 | 1.12 | 6.35 | 7.47             | 85        |
| Soils of the unaffected area |         |                          |           |           |               |      |      |      |      |      |      |                  |           |
| Ap                           | 0-30    | 5.53                     | 0.8       | 0.062     | 0.36          | 0.3  | 0.27 | 3    | 3.2  | 1.21 | 6.77 | 7.98             | 84.8      |
| B                            | 30-45   | 5.8                      | 0.8       | 0.056     | 0.3           | 0.29 | 0.26 | 2.9  | 3    | 1.21 | 6.45 | 7.57             | 85.2      |
| Bt1                          | 45-110  | 5.97                     | 0.38      | 0.06      | 0.38          | 0.34 | 0.32 | 3.2  | 3.42 | 1.1  | 7.28 | 8.38             | 86.9      |
| Bt2                          | 110-139 | 5.74                     | 0.08      | 0.063     | 0.32          | 0.32 | 0.3  | 3.1  | 3.2  | 1    | 6.92 | 7.92             | 87.4      |
| BC                           | 139-169 | 6.06                     | 0.04      | 0.064     | 0.34          | 0.32 | 0.31 | 3    | 3    | 1.1  | 6.64 | 7.74             | 85.8      |

OC= organic carbon, TN= total nitrogen, Av.P= available phosphorus, EA = exchangeable acidity, TEB = total exchangeable bases, BS = base saturation, CEC = cation exchange capacity

The values of exchangeable cations (Ca, Mg, K and Na) were low in all the soil units (Table 1). The low values of exchangeable bases of these soils maybe due to intensity of weathering and lateral translocation of bases Igomu and Idoga (2017). The amount of exchangeable calcium (Ca) ranged between 3.01 to 3.42 and 3.00 cmol/kg to 3.42 cmol/kg of soil, Magnesium (Mg) ranged between 2.60 to 3.21 and 2.90 to 3.20 cmol/kg of soil, Potassium (K) ranged between 0.24 to 0.30 and 0.29 to 0.32 cmol/kg of soil while Sodium (Na) ranged between 0.30 to 0.36 and 0.26 to 0.32 cmol/kg of soils of affected and unaffected areas respectively. The predominance of Ca over the other cations in these soils may be because of all the exchangeable cations, Ca is least easily lost from the soil environment Odoemena and Igomu (2017). Table 1 further shows that, Exchangeable Potassium (K) and Magnesium (Mg) were slightly higher in soils of the unaffected area (Gaando community) than those of the affected area (Tse-Kucha community) but the differences were insignificant with p-values of 0 and 0.01. Table 2 also shows that, there is no significant difference between the soils of the two study areas in terms of exchangeable Calcium (Ca) and Sodium (Na) content with p-values of zero (0) each. Exchangeable acidity was slightly higher in soils of the affected area than those of the unaffected area as shown in Table 1 but the difference was insignificant as shown in Table 2.

**Table 2. Summary of statistical analysis**

| Soil variables        | Mean  | SD     | Mean  | SD     | t-value             | p-value |
|-----------------------|-------|--------|-------|--------|---------------------|---------|
| pH (H <sub>2</sub> O) | 6.66  | 9.99   | 5.82  | 10.41  | 158*                | 0.01    |
| OC (%)                | 1.15  | 1.71   | 0.42  | 0.75   | 0.794*              | 0.01    |
| TN (%)                | 0.06  | 0.09   | 0.06  | 0.11   | 0.154 <sup>NS</sup> | 0       |
| Av.P (mg/kg)          | 0.34  | 0.5    | 0.34  | 0.61   | 0.370 <sup>NS</sup> | 0       |
| K (coml/kg)           | 0.27  | 0.41   | 0.31  | 0.56   | 0.003 <sup>NS</sup> | 0       |
| Na (coml/kg)          | 0.32  | 0.48   | 0.3   | 0.52   | 0.043 <sup>NS</sup> | 0       |
| Mg (coml/kg)          | 2.9   | 4.36   | 3.04  | 5.44   | 0.129*              | 0.01    |
| Ca (coml/kg)          | 3.18  | 4.78   | 3.17  | 5.66   | 0.089 <sup>NS</sup> | 0       |
| EA (coml/kg)          | 1.06  | 1.59   | 1.11  | 1.98   | 0.042 <sup>NS</sup> | 0       |
| TEB (coml/kg)         | 6.68  | 10.02  | 6.81  | 12.19  | 0.018 <sup>NS</sup> | 0       |
| CEC (coml/kg)         | 7.74  | 11.6   | 7.92  | 14.16  | 0.021 <sup>NS</sup> | 0       |
| BS (%)                | 86.25 | 129.38 | 86.02 | 153.88 | 2.435*              | 0.01    |

OC= organic carbon, TN= total nitrogen, Av.P= available phosphorus, EA = exchangeable acidity, TEB = total exchangeable bases, BS = base saturation, CEC = cation exchange capacity, SD= standard deviation

There was a slight difference in cation exchange capacity between the two study areas. It was just slightly higher in the unaffected soils. This could be due to the presence of more exchangeable cations and amount of clay in the soil. But Table 2 shows that there was no significant difference between the soils of the two study areas in terms of cation exchange capacity. Odoemena

and Igomu (2017) reported that some sandy soils of the tropics with dominant sesquioxide clay might have low CEC hence, low fertility.

The percentage base saturation was very high according to the ratings of Landon (1991), among the pedons. There is also no significant difference between the soils of the two study sites in terms of percentage base saturation and it ranges from 84.80 % to 87.90 % across the two study sites which is suitable for crop production.

### Conclusions

Presently, the cement dust from the factory does not significantly affect the chemical properties of soils of the study area (Tse–Kucha Community) within which the factory is located as the dust deposits only led to a slight but insignificant change in a few chemical properties but the variation is not significant enough to adversely affect crop production. This agrees with the findings of Asadu and Agada, (2008).

Furthermore, the cement dust from the factory enhanced some of the selected chemical properties such as pH, exchangeable acidity and percentage base saturation and may manifest positively on crop performance around the area in the future. Hence if properly harnessed may contribute to soil fertility management in the affected areas. This is also in line with the research findings of Asadu and Agada, (2008) who also stated that the highest quantity of cement dust obtained in farm plots was only about 0.72t/ha/yr which is too small to cause any negative effects on soils and crops. This is because the company now has a way of collecting the dust within the factory instead of allowing it to spread out unlike in the past when the dust was being allowed to spread out.

### Recommendations

Based on these research findings, the following management practices are recommended:

- ✓ Incorporation of crop residues to provide organic materials for the soil.
- ✓ Due to the low concentrations of Nitrogen in soils of the two study sites, farmers should adopt the use of high nitrogen fertilizers.
- ✓ There should also be installation of more dust collection equipment to further reduce dust emission during quarrying and manufacture of cement.
- ✓ There should also be an alternative use of dust trappings in air pollution control devices. It could be recycled for soil amendment for agricultural and other purposes.

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# Pig Slurry Influence on Soil Physio-Chemical Properties in Ngor Okpala, Imo State, Nigeria

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## Abstract

Recently, the importance of organic manure in enhancing soil physico-chemical properties cannot be overemphasized. Therefore a study was conducted to investigate pig slurry influence on soil physico-chemical properties in Ngor Okpala Imo State Nigeria. The experiment was laid out in a Randomized Complete Block Design (RCBD) with five (5) treatments replicated four (4) times. Data obtained was analyzed using analysis of variance (ANOVA) and significant means were separated using Fisher least significant difference (F-LSD) at probability level of ( $P=0.05$ ). The treatments consisted of P0-control. P1- pig slurry at  $10 \text{ t ha}^{-1}$ , P2- pig slurry at  $20 \text{ t ha}^{-1}$ , P3- pig slurry at  $30 \text{ t ha}^{-1}$  and P4- pig slurry at  $40 \text{ t ha}^{-1}$ . The result showed that application of pig slurry at  $10 \text{ t ha}^{-1}$ ,  $20 \text{ t ha}^{-1}$ ,  $30 \text{ t ha}^{-1}$  and  $40 \text{ t ha}^{-1}$  significantly ( $P= 0.05$ ) improved the soil total porosity, moisture content and decreased the soil bulk density. Also the application of pig slurry at different rates improved the soil  $\text{pH}_w$ , organic carbon, total nitrogen, available phosphorous, exchangeable bases, Effective cation exchange capacity (ECEC) and percentage base saturation and significantly decreased the soil total exchangeable acidity. The trend of improving the soil physico-chemical properties was  $40 \text{ t ha}^{-1}$ ,  $30 \text{ t ha}^{-1}$ ,  $20 \text{ t ha}^{-1}$ ,  $10 \text{ t ha}^{-1}$  and  $0 \text{ t ha}^{-1}$ . Therefore, it is recommended that farmers should use pig slurry in improving soil physico-chemical properties particularly at  $40 \text{ t ha}^{-1}$  rate of application.

**Keywords:** influence, chemical, physical, pig slurry, soil

## Introduction

Soil nutrient management is necessary to maintain the constant productivity of crops as well as good quality soil. The application of manure in the soil improves its fertility and increases agricultural production (Yolanda *et al.*, 2016). The use of mineral fertilizer and organic manure to improve soil quality has both negative and positive effect on plant growth and soil (Si Ho Han *et al.*, 2016). Continuous use of mineral fertilizer for increasing soil nutrient is usually accompanied on the long term by reduction in soil organic matter, erosion, degradation of soil physical properties and decrease in soil pH (Cai *et al.*, 2015). Mineral fertilizer could also negatively impact the microbial diversity by altering the bacterial and fungal population (Asmita, 2019).

Organic manure on the other hand has shortcomings which include slow decomposition and low nutrient depending on the nutrient composition of the organic material (Si Ho Han *et al.*, 2016).



However, organic manure is considered less likely to have detrimental effects on soil physical and chemical properties compared with mineral fertilizer (Soremi *et al.*, 2017). Therefore, replacement of mineral fertilizer with organic manure can be an alternative to enhance soil quality and crop growth (Jiang *et al.*, 2018).

The application of organic manure to the soil has positive effect on soil physio-chemical properties mainly due to increase in organic matter (Yolanda *et al.*, 2014). The vital roles of organic matter include being a rich source of essential plant nutrients, helps in improving water holding capacity of soil, improves soil structure, soil aeration, water permeability, acts as pH buffer, contains metal organic matter complexes that help in making available micro nutrients to crops (Ikeh *et al.*, 2013, Soremi *et al.*, 2017).

Studies have shown that application of organic manure to the soil helps to increase soil quality and productivity by its own favorable effects on soil properties (Ozlu and Kumar, 2018). Ye *et al.*, (2019) also reported an increase in soil organic matter due to application of organic manure. Increase in soil organic matter can enhance aggregate stability; improve soil structure, increase nutrient cycling, microbial diversity and enzyme activity (Lupwayi *et al.*, 2019). Application of manure improve soil structure leading to high porosity, water holding capacity and water infiltration as well as maintains soil pH (Ozlu and Kumar, 2018). The availability of nutrients to crops is affected by the physio-chemical and biological properties of soil such as pH, organic matter, soil texture, nutrient interactions, soil moisture content, temperature and microbial activities Soremi *et al.*, 2017). Processes in the soil such as mineralization, humification, adsorption, desorption, precipitation, immobilization, leaching, diffusion and fixation could be triggered within the soil environment by the addition of organic manure which leads to changes in soil physio-chemical properties and resultant in optimum growth of crops. Therefore manure application is being promoted as an alternative to mineral fertilizer to enhance agricultural sustainability (Gai *et al.*, 2019).

The objective of this study was to investigate the influence of pig slurry on soil physio-chemical properties in Ngor Okpala Imo State Nigeria.

## Materials and Methods

### Site Description

The study was carried out at Umueme Obike in Ngor Okpala L.G.A, Imo State Nigeria. The study site is located on latitude 5°18'50.352"N and Longitude 7°8'34.062"E. The study site had a humid tropical climate, annual temperature of 27-29° and annual rainfall of 1500m-2500m which starts from March to December with its peak in July. The dry season starts from December to March with a dry dust and cold intervals. The vegetation consists of mostly grasses such as guinea grass (*Panicum maximum*), elephant grass (*Pennisetum purpureum*) and star grass (*Cynodon nlemfuensis*).

### Site Preparation/Treatment Allocation

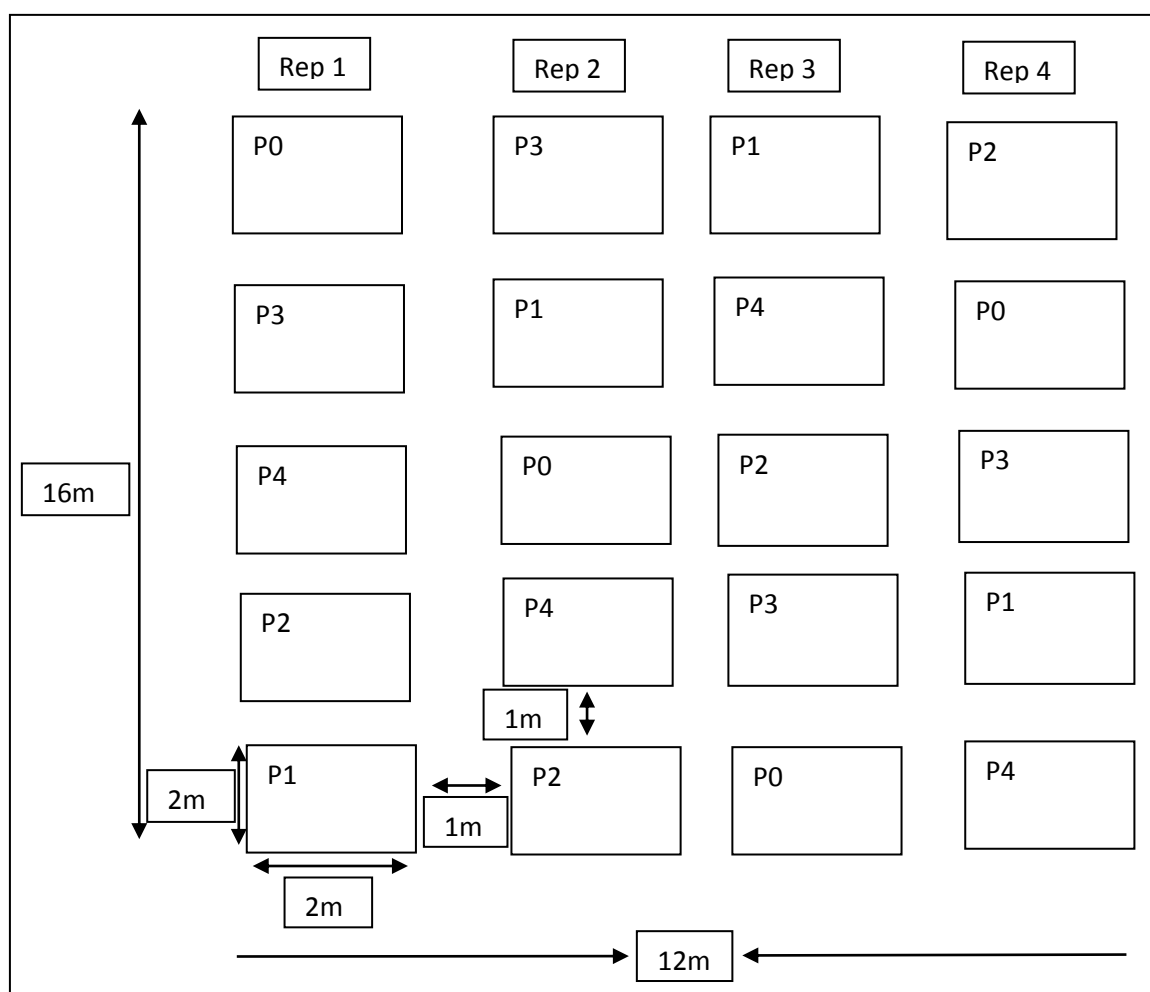
A total land area of 192m<sup>2</sup> (16×12) was used for the experiment. The experimental site was manually cleared using machetes, spade and hoe. Experimental plots were mapped out using Measuring tapes, pegs and ropes. Twenty (20) experimental plots were used for the study, measuring 2m×2m with 1m apart to separate the treatment effects from interfering with each other. The treatments comprised of P0- control, P1- Pig slurry at 10t ha<sup>-1</sup>, P2- Pig slurry at 20t ha<sup>-1</sup>, P3- Pig slurry at 30t ha<sup>-1</sup>, P4- Pig slurry at 40t ha<sup>-1</sup>.

### Sourcing of Treatment Material

Pig slurry was sourced from Amici Ranch Integrated Farm Umueme Ngor Okpala, Imo State Nigeria.

### Experimental Design

The experimental design used for the experiment was randomized complete block design (RCBD). The design consisted of five (5) treatments replicated four times as shown in figure 1.



**Figure 1.** Field layout of the Experimental Site

### Soil Sampling

Soil samples were collected at 0-20 cm soil depth for soil physical and chemical properties using core samplers and soil Augar. The samples in the core samplers were used for the determination of soil physical properties. Samples collected were stored in polyethene bags and sent to the soil handling unit of FUTO laboratory for processing which include air drying, crushing and sieving using 2mm sieve in preparatory for routine analysis.

### Laboratory Analysis

The laboratory soil analysis was carried out at Soil Science Laboratory of Federal University of Technology Owerri, Imo State and the following soil properties were determined; Organic

carbon was determined by the Walkley and Black (1934) wet-oxidation method as modified and described in manual of soil, plant and water analysis (Eno *et al.*, 2009) and the value for organic matter was obtained by multiplying organic carbon value by 1.724 (Van Bemmelen factor). Available phosphorous was determined by Bray and Kurtz No.2 (1945) method adopted by Juo (1979) in which the phosphorous was extracted with 1ml  $\text{NH}_4\text{F}$  and 0.5ml Hydrochloric acid. Colour development was achieved by adding “reagent B”. Available phosphorous was determined calorimetrically using a photoelectric calorimeter. Total nitrogen was determined by micro Kjeldahl digestion and distillation method of Bremner (1965) as recently modified and described in the manual of soil and water analysis (Eno *et al.*, 2009) using concentrated  $\text{H}_2\text{SO}_4$  and a sodium copper sulphate catalyst mixture. Soil pH was determined in the laboratory with a glass pH meter at a ratio of 1:2.5 water ratios, exchangeable cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$  and  $\text{K}^+$ ) were extracted with ammonium acetate solution, Exchangeable calcium and magnesium was determined by the ethylenediaminetetracetic acid titration method as described by Black (1965), whereas exchangeable potassium and sodium were determined by flame photometric method. Exchangeable acidity was determined by the titrimetric method described by Bray and Weit (1999). Total exchangeable acidity was determined by the summation of  $\text{H}^+$  and  $\text{Al}^{3+}$ . Total exchangeable bases were determined by the summation of exchangeable cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$  and  $\text{K}^+$ ). Effective Cation Exchange Capacity was determined by the summation of exchangeable acidity ( $\text{Al}^{3+}$  and  $\text{H}^+$ ) and exchangeable bases. Percentage base saturation (BS) was calculated by the summation of the total exchangeable bases (TEB) divided by effective cation exchange capacity (ECEC) and then multiplied by 100.

$$\% \text{ BS} = \frac{\text{TEB}}{\text{ECEC}} \times 1000 \quad \text{Equation}$$

(1)

The Selected Physical Properties determined were; Bulk density was determined using core method (Black and Hartge, 1986).

$$\text{Bulk Density} = \frac{\text{Weight of wet soil} - \text{Weight of dry soil}}{\text{Weight of wet soil}} \times 100 \quad \text{Equation}$$

(2)

Total porosity was calculated from the result of bulk density and particle size density. Total

$$\text{Porosity} = \frac{1 - \text{Bulk Density (g/cm}^3\text{)}}{\text{Particle Density}} \times 100 \quad \text{Equation (3)}$$

Particle Size Distribution was determined by hydrometric meter in water and Calgon (Bouyocous, 1951). Soil texture was determined by matching the value of the particle size against the textural triangle. Gravimetric moisture content will be determined by the gravimetric method calculated mathematically as follows:

$$\% \text{ MC} = \frac{W_2 - W_3}{W_3 - W_1} \times 100 \quad \text{Equation (4)}$$

Where MC = Moisture Content,  $W_1$  = Weight of the core,  $W_2$  = Weight of wet sample + core,  $W_3$  = Weight of oven – dried sample + core.

### Statistical Analysis

Data collected were analyzed using analysis of variance (ANOVA) with the aid of IBM SPSS statistics, version 23. The differences in mean were separated using Fishers Least Significant difference (FLSD) at  $P = 0.05$ .

## Results and Discussion

### Initial Soil Characteristics before Treatment Application

The initial physical and chemical properties of the soil before application of treatment are presented in Table 1

**Table 1.** Initial Soil Analysis

| Soil Properties and Units of Measurement                     | Value      |
|--------------------------------------------------------------|------------|
| <b>Physical properties</b>                                   |            |
| Sand (%)                                                     | 70.00      |
| Silt (%)                                                     | 42.40      |
| Clay ((%)                                                    | 22.20      |
| Textural class                                               | Loamy soil |
| Bulk Density ( $\text{g cm}^{-3}$ )                          | 1.51       |
| Moisture Content (%)                                         | 7.02       |
| Total Porosity (%)                                           | 37.9       |
| <b>Chemical properties</b>                                   |            |
| pH in $\text{H}_2\text{O}$ (1:2:5)                           | 5.25       |
| Organic Carbon (%)                                           | 1.35       |
| Available phosphorous $\text{mg kg}^{-1}$                    | 12.6       |
| Total Nitrogen (%)                                           | 0.12       |
| Calcium ( $\text{Cmol kg}^{-1}$ )                            | 1.40       |
| Magnesium ( $\text{Cmol kg}^{-1}$ )                          | 0.31       |
| Sodium ( $\text{Cmol kg}^{-1}$ )                             | 0.07       |
| Potassium ( $\text{Cmol kg}^{-1}$ )                          | 0.05       |
| Exchangeable acidity ( $\text{Cmol kg}^{-1}$ )               | 1.39       |
| Total Exchangeable Base ( $\text{Cmol kg}^{-1}$ )            | 1.83       |
| Effective Cation Exchange Capacity ( $\text{Cmol kg}^{-1}$ ) | 3.22       |
| Percentage Base Saturation (%)                               | 56.83      |

The result on the analysis of the initial soil characteristics before pig slurry application on soil physical and chemical properties is shown in Table 1. The result showed that the soil of the experimental site is loamy soil, placing the values of sand, silt and clay on a textural triangle according to the United State Department of Agriculture (USDA). The bulk density recorded  $1.51 \text{ g cm}^{-3}$  which could be as a result of the anthropogenic activities being carried out on the site, although it was within the tolerable limit of  $1.85 \text{ g cm}^{-3}$  for plant growth (FAO 1996). The total porosity was high with value of 38.95%, this increase in total porosity could be as a result of the bulk density because the lower the bulk density the higher the soil porosity. The  $\text{pH}_w$  was slightly acidic (5.25) which is good for retaining plant nutrients. The soil organic carbon was low; the minimal threshold for soil organic carbon in tropical soils is 2% (Patrick *et al.*, 2013). Total nitrogen was low with

value of 0.12% and available phosphorous was moderate with value of 12.6 mg kg<sup>-1</sup>. The result also recorded total exchangeable acidity (TEA) 1.39 Cmol kg<sup>-1</sup>, total exchangeable bases (TEB) of 1.83 Cmol kg<sup>-1</sup>, effective cation exchange capacity (ECEC) of 3.22 Cmol kg<sup>-1</sup> and percentage base saturation of 56.83%. Therefore these results indicated that the soil of the experimental site is low in soil fertility, hence will be improved by addition of organic input like pig slurry for optimum crop production.

### **Chemical Composition of Pig Slurry Used for the Experiment**

The result on the chemical composition of pig slurry used in the experiment is shown in Table 2.

The result showed that the soil pH<sub>w</sub> recorded 6.40 which is adequate for plant growth. The organic matter content was high with value of 28.2%. Total nitrogen and available phosphorous were high. The exchangeable bases calcium, magnesium, potassium and sodium were also high. This suggests that pig slurry is rich in mineral nutrients and would improve soil properties when added to the soil.

**Table 2.** Chemical Composition of Pig Slurry Used for the Experiment

| Soil Parameters/Minerals                     | Values |
|----------------------------------------------|--------|
| pH in H <sub>2</sub> O (1:2:5)               | 6.40   |
| Organic Carbon (%)                           | 16.4   |
| Organic Matter (%)                           | 28.2   |
| Total Nitrogen (%)                           | 6.80   |
| Available phosphorous (mg kg <sup>-1</sup> ) | 10.60  |
| Calcium (Cmol kg <sup>-1</sup> )             | 9.42   |
| Magnesium (Cmol kg <sup>-1</sup> )           | 3.90   |
| Potassium (Cmol kg <sup>-1</sup> )           | 5.21   |
| Sodium (Cmol kg <sup>-1</sup> )              | 1.30   |

### **The Influence of Pig Slurry on Soil Physical Properties**

The results of the influence of pig slurry on soil physical properties of the experimental site are shown in Table 3.

#### **Particle size distribution**

The result on pig slurry application on particle size distribution showed that there were no significant differences in % sand, % silt and % clay of the treated plots although there were variations in values. This could be attributed to the fact that the soil of the experimental site originated from the same parent material. The application of pig slurry at 10 t ha<sup>-1</sup> and 20 t ha<sup>-1</sup> increased in percentage sand, silt and clay content compared to the control. Also the application of pig slurry at 30 t ha<sup>-1</sup> reduced in % sand, silt and clay compared to P1 (10 t ha<sup>-1</sup>) and P2 (20 t ha<sup>-1</sup>). However, P4 (40 t ha<sup>-1</sup>) recorded the highest % sand, silt and clay.

#### **Bulk Density (g cm<sup>-3</sup>)**

The Soil bulk density decreased with application of pig slurry. The different levels of pig slurry added to the soil recorded different significant (P=0.05) results. There was significant difference when the control was compared with the treatments and when the treatments were

compared to each other. The untreated plot (control) recorded 0.05, 0.11, 0.12 and 0.13 g cm<sup>-3</sup> increase in bulk density compared to 10 t ha<sup>-1</sup>, 20 t ha<sup>-1</sup>, 30 t ha<sup>-1</sup> and 40 t ha<sup>-1</sup> treated plots. 10 t ha<sup>-1</sup> amended plot recorded 0.06, 0.07 and 0.08 g cm<sup>-3</sup> increase in bulk density compared to 20 t ha<sup>-1</sup>, 30 t ha<sup>-1</sup> and 40 t ha<sup>-1</sup> respectively. 20 t ha<sup>-1</sup> recorded 0.02 and 0.03 g cm<sup>-3</sup> increase in bulk density than 30 t ha<sup>-1</sup> and 40 t ha<sup>-1</sup>. However, there was no significant difference between 30 t ha<sup>-1</sup> and 40 t ha<sup>-1</sup> treated plots. The soil bulk density decreased with an increasing rate of pig slurry application. This decrease in soil bulk density of the amended plots could be attributed to the increased soil organic matter of pig slurry treatment. This supports the findings of Adekiya *et al.*, (2016), who noted that organic amendments added to the soil reduced soil bulk density due to the enhancement of soil organic matter by the amendment.

**Table 3. Influence of Pig Slurry on Soil Physical Properties**

| Treatment | Sand  | Silt  | Clay  | Textural Class | BD                | TP                 | MC                |
|-----------|-------|-------|-------|----------------|-------------------|--------------------|-------------------|
|           | (%)   | (%)   | (%)   |                | gcm <sup>-3</sup> | (%)                | (%)               |
| P0        | 70.14 | 41    | 22    | loamy Soil     | 1.52 <sup>d</sup> | 38.95 <sup>a</sup> | 6.98 <sup>a</sup> |
| P1        | 71.51 | 42    | 23.1  | loamy Soil     | 1.47 <sup>c</sup> | 45.83 <sup>b</sup> | 8.08 <sup>b</sup> |
| P2        | 71.58 | 42    | 24.11 | loamy Soil     | 1.42 <sup>b</sup> | 47.49 <sup>c</sup> | 8.10 <sup>b</sup> |
| P3        | 70.25 | 39.72 | 22.1  | loamy Soil     | 1.40 <sup>a</sup> | 48.13 <sup>d</sup> | 8.41 <sup>c</sup> |
| P4        | 72    | 40    | 23.2  | loamy Soil     | 1.39 <sup>a</sup> | 48.47 <sup>d</sup> | 8.57 <sup>d</sup> |
| F-LSD     | NS    | NS    | NS    |                | 0.01              | 0.73               | 0.182             |
| -0.05     |       |       |       |                |                   |                    |                   |

**Legend:** P0 = Control, P1 = Pig Slurry at 10 t ha<sup>-1</sup>, P2 = Pig Slurry at 20 t ha<sup>-1</sup>, P3 = Pig Slurry at 30 t ha<sup>-1</sup>, P4 = Pig Slurry at 40 t ha<sup>-1</sup>, BD = Bulk density, TP = Total porosity, MC=Moisture Content.

**Note:** Figures with the same superscript are not statistically significantly different.

### Total Porosity (%)

The influence of pig slurry application on soil total porosity showed that there was a significant  $P = (0.05)$  increase in soil total porosity when the control was compared with the treatments. The control recorded 6.88, 8.54, 9.18, and 9.58 % less total porosity compared to 10 t ha<sup>-1</sup>, 20 t ha<sup>-1</sup>, 30 t ha<sup>-1</sup> and 40 t ha<sup>-1</sup> treated plots respectively. Also there was a significant difference when different rates of pig slurry application were compared with one another. The 10 t ha<sup>-1</sup> rate application recorded 1.66, 2.30 and 2.63 % less total porosity compared with 20 t ha<sup>-1</sup>, 30 t ha<sup>-1</sup>, and 40 t ha<sup>-1</sup> rates. There was no significant difference in soil total porosity when 30 t ha<sup>-1</sup> was compared with 40 t ha<sup>-1</sup>, however the values differs. The increment in soil total porosity in the amended plots could be as a result of decreased bulk density of the amended plots.

### Moisture Content (%)

The soil moisture content improved with addition of pig slurry at different rates. The control plot recorded 1.10, 1.12, 1.43 and 1.59 % less moisture content compared with 10 t ha<sup>-1</sup>, 20 t ha<sup>-1</sup>, 30 t ha<sup>-1</sup>, and 40 t ha<sup>-1</sup> respectively. There was no significant  $P = (0.05)$  difference when 10 t ha<sup>-1</sup> pig slurry application rate was compared with 20 t ha<sup>-1</sup> rate of application. 20 t ha<sup>-1</sup> recorded 0.31 and 0.47 % less moisture content than 30 t ha<sup>-1</sup> and 40 t ha<sup>-1</sup> respectively. The 40 t ha<sup>-1</sup> pig slurry treated plot recorded 0.16% more moisture content compared with 30 t ha<sup>-1</sup>. Application of pig slurry at



different rates improved the soil moisture content from 6.98 – 8.57%. This improvement in soil moisture content with levels of pig slurry application is attributed to the mulching effect of organic manure and improved moisture retention and water acceptance as a result of improved soil structure and micro porosity (Adekiya *et al.*, 2016).

### **The Influence of Pig Slurry on Soil Chemical Properties**

The results of the influence of pig slurry application on soil chemical properties of the experimental site are shown in Table 4.

#### **Soil pH<sub>w</sub>**

Pig slurry influence on soil pH showed that there were significant ( $P = 0.05$ ) difference when the control was compared with treatments at 10 t ha<sup>-1</sup>, 20 t ha<sup>-1</sup>, 30 t ha<sup>-1</sup>, and 40 t ha<sup>-1</sup>. The control decreased the pH of the soil with value of 0.84, 0.93, 1.12, and 1.66 compared with p1 (10 t ha<sup>-1</sup>), p2 (20 t ha<sup>-1</sup>), p3 (30 t ha<sup>-1</sup>), and p4 (40 t ha<sup>-1</sup>) respectively. Also when the treatments were compared with one another, Soil pH increased with an increasing rate of pig slurry application. 40 t ha<sup>-1</sup> rate improved the soil pH tremendously. There was a strong shift from acidity to slight acidic. The improvement in soil pH in the amended plots could be due to the liming effect of agro wasted as reported by Manna *et al.*, (2007). Also Soromi *et al.*, (2017) reported an increase in soil pH as a result of the release of basic cations by organic matter due to mineralization.

#### **Organic Carbon**

Pig slurry application significantly ( $P = 0.05$ ) improved soil organic carbon when the control was compared with the treated plots at 10 t ha<sup>-1</sup>, 20 t ha<sup>-1</sup>, 30 t ha<sup>-1</sup>, and 40 t ha<sup>-1</sup>. Application of pig slurry increased soil organic carbon by 0.05, 0.38, 0.73 and 1.18% respectively. Also there was significant difference in soil organic carbon content of the treated plots. 10 t ha<sup>-1</sup> recorded 0.33, 0.68 and 1.13 less percentage organic carbon content compared to 20 t ha<sup>-1</sup>, 30 t ha<sup>-1</sup>, and 40 t ha<sup>-1</sup> rates. However the highest organic carbon content of the treated plots was achieved in pig slurry application at 40 t ha<sup>-1</sup>. This increase in soil organic carbon content could be attributed to the release of organic carbon contained in pig slurry during mineralization. This is in line with the consistent findings of NRC (1996) who noted that applying animal manure increases the supply of organic carbon hence organic matter in the soil. Also the application of organic manure in general increases soil organic carbon (Ozlu and Kumar, 2018).

#### **Total Nitrogen**

The soil total nitrogen improved with pig slurry application at different rates compared with the control. There were also significant ( $P = 0.05$ ) improvement in soil total nitrogen when the treatments were compared with one another. The control plot recorded 0.07, 0.08, 0.13 and 0.12% less total nitrogen compared with pig slurry treated plots at different rates respectively. Also there was an improvement in soil total nitrogen when the treated plots were compared with one another. The 10 t ha<sup>-1</sup> recorded 0.01, 0.06 and 0.17 less nitrogen compared with 20 t ha<sup>-1</sup>, 30 t ha<sup>-1</sup> and 40 t ha<sup>-1</sup> treated plots respectively. The highest improvement was recorded in plots treated with 40 t ha<sup>-1</sup> followed by 30 t ha<sup>-1</sup> and 20 t ha<sup>-1</sup>. This improvement in soil total nitrogen by application of pig slurry could be as a result of the mineralization of organic wastes such as pig slurry which resulted in the release of organic bound nutrients in the soil notably NPK and organic matter. (Ekpe *et al.*, 2017)

**Table 4.** Influence of Pig Slurry on Soil Chemical Properties

| TRET         | P <sup>H</sup> <sub>w</sub> | OC (%)            | N (%)             | AVP (mg/kg)        | Ca <sup>2+</sup>  | Mg <sup>2+</sup>  | K <sup>+</sup>    | Na <sup>+</sup>   | TEA               | TEB               | ECEC              | BS %               |
|--------------|-----------------------------|-------------------|-------------------|--------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|--------------------|
|              |                             |                   |                   |                    | → Cmol/kg         |                   |                   |                   | ←                 |                   |                   |                    |
| P0           | 5.25 <sup>a</sup>           | 1.58 <sup>a</sup> | 0.14 <sup>a</sup> | 18.84 <sup>a</sup> | 1.41 <sup>a</sup> | 0.36 <sup>a</sup> | 0.06 <sup>a</sup> | 0.05 <sup>a</sup> | 1.37 <sup>e</sup> | 1.88 <sup>a</sup> | 3.25 <sup>a</sup> | 57.84 <sup>a</sup> |
| P1           | 6.09 <sup>b</sup>           | 1.63 <sup>b</sup> | 0.21 <sup>c</sup> | 23.69 <sup>c</sup> | 1.67 <sup>b</sup> | 0.58 <sup>b</sup> | 0.08 <sup>a</sup> | 0.08 <sup>b</sup> | 1.32 <sup>d</sup> | 2.41 <sup>b</sup> | 3.73 <sup>b</sup> | 64.61 <sup>b</sup> |
| P2           | 6.18 <sup>c</sup>           | 1.96 <sup>c</sup> | 0.22 <sup>b</sup> | 25.56 <sup>b</sup> | 2.15 <sup>c</sup> | 0.72 <sup>c</sup> | 0.13 <sup>b</sup> | 0.09 <sup>b</sup> | 1.28 <sup>c</sup> | 3.09 <sup>c</sup> | 4.37 <sup>c</sup> | 70.70 <sup>c</sup> |
| P3           | 6.37 <sup>d</sup>           | 2.31 <sup>d</sup> | 0.27 <sup>d</sup> | 29.76 <sup>d</sup> | 2.22 <sup>d</sup> | 0.77 <sup>d</sup> | 0.18 <sup>c</sup> | 0.13 <sup>c</sup> | 1.24 <sup>b</sup> | 3.30 <sup>d</sup> | 4.54 <sup>d</sup> | 72.68 <sup>d</sup> |
| P4           | 6.91 <sup>e</sup>           | 2.76 <sup>e</sup> | 0.31 <sup>e</sup> | 30.08 <sup>e</sup> | 2.27 <sup>e</sup> | 1.12 <sup>e</sup> | 0.25 <sup>d</sup> | 0.15 <sup>d</sup> | 1.16 <sup>a</sup> | 3.79 <sup>e</sup> | 4.95 <sup>e</sup> | 76.56 <sup>e</sup> |
| F-LSD (0.05) | 0.0181                      | 0.021             | 0.006             | 0.410              | 0.028             | 0.046             | 0.022             | 0.006             | 0.011             | 0.020             | 0.031             | 0.151              |

**Key:** OC = Organic Carbon, OM = Organic Matter, N = Nitrogen, AVP = Available Phosphorous, Ca = Calcium, Mg = Magnesium, K = Potassium, Na = Sodium, H = Hydrogen, AL = Aluminum, TEA = Total Exchangeable Acidity, TEB = Total Exchangeable Bases, ECEC = Effective Cation Exchange Capacity, BS = Base Saturation, P0 = Control, P1 = Pig Slurry at 10 t ha<sup>-1</sup>, P2 = Pig Slurry at 20 t ha<sup>-1</sup>, P3 = Pig Slurry at 30 t ha<sup>-1</sup>, P4 = Pig Slurry at 40 t ha<sup>-1</sup>.  
**Note:** Figures with the same superscript are not statistically significant

### Available Phosphorous

The influence of pig slurry on available phosphorous showed that the application of pig slurry at 10 t ha<sup>-1</sup>, 20 t ha<sup>-1</sup>, 30 t ha<sup>-1</sup>, and 40 t ha<sup>-1</sup> significantly ( $P = 0.05$ ) increased the phosphorous content of the soil compared to the control. The control plot recorded 4.85, 6.72, 10.92 and 11.24 less phosphorous content to 10 t ha<sup>-1</sup>, 20 t ha<sup>-1</sup>, 30 t ha<sup>-1</sup>, and 40 t ha<sup>-1</sup> treated plots respectively. When the treatments at different rates were compared with one another, there were also significant ( $P = 0.05$ ) differences observed. The 40 t ha<sup>-1</sup> rate improved the soil phosphorous content by 6.39, 4.52 and 0.32 compared with 10 t ha<sup>-1</sup>, 20 t ha<sup>-1</sup> and 30 t ha<sup>-1</sup> treated plots respectively. However, the high content of available phosphorous at higher application rate of pig slurry application could be attributed to the effect of P content in pig slurry. Also the mineralization of organic P releases P into the soil solution contributing to the observed high available P content in the treated plots.

### Total Exchangeable Bases (Ca, Mg, K, Na)

The total exchangeable bases of the soil improved with the application of pig slurry. The different rates of pig slurry application also recorded different significant ( $P = 0.05$ ) results. There was significant difference when 10 t ha<sup>-1</sup> pig slurry applications was compared with 20 t ha<sup>-1</sup>, 30 t ha<sup>-1</sup>, and 40 t ha<sup>-1</sup>. Also there was significant difference when 20 t ha<sup>-1</sup> was compared with 30 t ha<sup>-1</sup> and 40 t ha<sup>-1</sup>. 30 t ha<sup>-1</sup> also differ significantly from 40 t ha<sup>-1</sup>. Treatment rates of 10 t ha<sup>-1</sup>, 20 t ha<sup>-1</sup>, 30 t ha<sup>-1</sup>, and 40 t ha<sup>-1</sup> recorded 0.53, 1.21, 1.42 and 1.91 Cmol kg<sup>-1</sup> more exchangeable bases compared with untreated plot (control). Also, 20 t ha<sup>-1</sup>, 30 t ha<sup>-1</sup>, and 40 t ha<sup>-1</sup> rates recorded 0.68, 0.89, and 1.38 Cmol kg<sup>-1</sup> higher exchangeable bases compared to 10 t ha<sup>-1</sup> rate. On the other hand, 40 t ha<sup>-1</sup> recorded 0.70 and 0.49 Cmol kg<sup>-1</sup> compared with 20 t ha<sup>-1</sup> and 30 t ha<sup>-1</sup>, therefore 40 t ha<sup>-1</sup> recorded the highest increase in total exchangeable bases. The increased total exchangeable bases recorded by the treated plots could be due to the increased soil pH caused by the addition of pig slurry which invariably has a liming effect on the soil and this result is inline with the findings of Aruna *et al.*, (2020).

### Total Exchangeable Acidity (TEA)

Pig slurry influence on total exchangeable acidity significantly reduced the acidity of the soil compared to the control. The treated plots (10 t ha<sup>-1</sup>, 20 t ha<sup>-1</sup>, 30 t ha<sup>-1</sup>, and 40 t ha<sup>-1</sup>) reduced the acidity of the soil by 0.05, 0.09, 0.13 and 0.21 Cmol kg<sup>-1</sup> respectively, when compared with control plot. Also when the treatments were compared with one another, significant differences were observed. The 40 t ha<sup>-1</sup> treated plot reduced the total exchangeable acidity significantly by 0.16, 0.12, 0.08 Cmol kg<sup>-1</sup> respectively when compared with 10 t ha<sup>-1</sup>, 20 t ha<sup>-1</sup> and 30 t ha<sup>-1</sup> treated plots. However, the soil total exchangeable acidity decreased significantly with an increasing rate of pig slurry application. This decrease in total exchangeable acidity could be attributed to the increased total exchangeable bases. This result supports the findings of Edeh *et al.*, (2015) who noted a decrease in soil total exchangeable acidity due to the addition of organic waste.

### Effective Cation Exchange Capacity (ECEC)

There was significant difference in soil ECEC when the control plot was compared with the treated plots. The control plot recorded significantly 0.48, 1.12, 1.29 and 1.70  $\text{Cmol kg}^{-1}$  less ECEC compared with 10  $\text{t ha}^{-1}$ , 20  $\text{t ha}^{-1}$ , 30  $\text{t ha}^{-1}$ , and 40  $\text{t ha}^{-1}$  respectively. Also there was significant difference when the different treatment levels of pig slurry were compared with one another. 20  $\text{t ha}^{-1}$ , 30  $\text{t ha}^{-1}$ , and 40  $\text{t ha}^{-1}$  recorded 0.64, 0.81 and 1.22  $\text{Cmol kg}^{-1}$  more ECEC compared to 10  $\text{t ha}^{-1}$  treated plot. 30  $\text{t ha}^{-1}$  recorded 0.17  $\text{Cmol kg}^{-1}$  over 20  $\text{t ha}^{-1}$  and 40  $\text{t ha}^{-1}$  recorded 0.41  $\text{Cmol kg}^{-1}$  over 30  $\text{t ha}^{-1}$ . The soil ECEC of the amended plots increased with an increasing rate of pig slurry application with the range 3.73-4.95  $\text{Cmol kg}^{-1}$ . This indicates that organic matter retains nutrients by providing cation and anion exchange capacity. This supports the findings of NRC, (1996). Similar findings on the increase in soil ECEC was reported by Ojobor *et al.*, (2017).

### Base Saturation (%)

There was significant difference in the soil % base saturation when the result of the control was compared with the results of the amended plots and when the treatments levels were compared with one another. The control recorded 6.77, 12.86, 14.84 and 18.72 % less base saturation compared to 10  $\text{t ha}^{-1}$ , 20  $\text{t ha}^{-1}$ , 30  $\text{t ha}^{-1}$  and 40  $\text{t ha}^{-1}$  respectively. Also 10  $\text{t ha}^{-1}$  treated plot recorded 6.09, 8.07 and 11.95 % less base saturation compared to 20  $\text{t ha}^{-1}$ , 30  $\text{t ha}^{-1}$ , and 40  $\text{t ha}^{-1}$ . The 30  $\text{t ha}^{-1}$  treated plot recorded 1.98 and 3.88 less base saturation compared to 40  $\text{t ha}^{-1}$ . However, there was superior improvement in soil percentage base saturation by the amended plots than the control plot with the highest increase in soil base saturation recorded in 40  $\text{t ha}^{-1}$  treated plot. This improvement in the amended plots supports the findings of Saoussan *et al.*, (2020).

### Conclusion

In this study, the results obtained had shown that pig slurry application at 10  $\text{t ha}^{-1}$ , 20  $\text{t ha}^{-1}$ , 30  $\text{t ha}^{-1}$  and 40  $\text{t ha}^{-1}$  improved soil physio-chemical properties and can be recommended as a good source of organic manure for improving soil properties. 40  $\text{t ha}^{-1}$  was the best rate of application compared to 10  $\text{t ha}^{-1}$ , 20  $\text{t ha}^{-1}$  and 30  $\text{t ha}^{-1}$  rates.

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# Utilization of wood ash as soil amendment and fertilizer on some growth and physiological indices of *Capsicum annum* (pepper)



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## Abstract

Sustainable utilization of wastes such as wood ash generated by small scale industries is an innovative method of waste management. This study elucidated effects of varying levels (250 mL WALF + 750 Water, 500 mL WALF + 500 mL Water, 750 mL WALF + 250 mL Water, 1000mL WALF) of inclusion of wood ash liquid fertilizer (WALF) on cultivation of *C. annum* (pepper). Distilled water served as control. Agronomical and physiological parameters of the pepper were determined. Height (18.82 cm), number of leaf (16.60), leaf breadth (3.58 cm), and stem girth (0.90 cm) of the pepper treated with combination 500 mL WALF + 500 mL Water were significantly higher compared with other combinations of the WALF. Similar observation was noticed in chlorophyll ( $13.6 \mu\text{mol m}^{-2}$ ) contents determined in the leaves of the pepper treated with the same combination. Higher net assimilation rate ( $0.80 \text{ gm}^{-2}\text{day}^{-1}$ ), relative growth rate ( $0.78 \text{ mgg}^{-1}\text{day}^{-1}$ ), leaf area ratio ( $0.08 \text{ m}^2\text{kg}^{-1}$ ), leaf area (83.56 cm) and specific leaf area ( $142.56\text{m}^2.\text{kg}^{-1}$ ) were observed in the leaves of the pepper treated with 500 mL WALF + 500 mL Water. In conclusion the 500 mL WALF + 500 mL Water enhanced better the agronomic and physiological parameters of the vegetable, therefore, the combination is recommended for sustainable cultivation of pepper.

**Keywords:** growth component, wood ash, liquid fertilizer, waste management, morphological character, vegetables, innovation

## Introduction

Management of industrial wastes has significant effects on survival of flora and fauna found in our environment. The effects therefore motivate the sustainable usage of plant-based wastes to enhance production of vegetables such as *Capsicum annum* as one the commonly cultivated peppers. *C. annum* is an annual tropical vegetable grown for its sensory attributes and for preservation of agricultural products in organic farming systems (Ademoyegu et al., 2011; Idowu-Agida et al., 2012, Sanusi and Ayinde, 2013). Despite the economic importance of the vegetable, there is low production of the crop due to poor land practice, insufficient fertilizers or high cost of inorganic fertilizers, inappropriate quantity of the fertilizers and their possible deleterious effects on soil microorganism if applied inappropriately.

Over the years, over cropping or intensive cultivation of vegetables including *C. annum* on certain areas of land most especially in urban areas has resulted into depletion of soil nutrients and increase in soil acidity. Incidentally, the supply of organic manure is not enough, especially in urban areas where agricultural land is limited in nutritional contents to substitute the use of chemical fertilizer and as a quick means of nutrient replenishment. Therefore, application of organic fertilizer such as wood ash may help in coping with the low level of nutrients in the soil (Witt et al., 2005). According to Okore et al. (2012), vegetables and spices such as pepper take up large amount of nutrients from the soil hence the need for replacement of such soil with organic substances through introduction of plant-based wastes such as wood ash (Ademoyegun et al., 2011) to augment mineral elements depleted in the soil (Jacobson, 2001, Adekayode and Olojugba, 2010).

In the recent times, there has been a clarion call for sustainable utilization of natural resources in farming as biotechnological approach towards recycling to meet up with food demands of geometric increase in population, gradual reduction or extinction of natural resources. Lack of agricultural inputs such as fertilizers contributed largely to low farm produce, less quality of produce and increase in food insecurity in Nigeria.

In addition, many local farmers cannot afford the exorbitant prices of synthetic fertilizers talk less of preventing their adverse effects on the produce. Therefore, there is urgent need to adopt the usage of organic fertilizer of plant origin such as wood ash as source of soil amendment. Disposal of wood ash as a product of complete combustion of timber is an environmental threat especially in an area where small scale industries such as bakeries, charcoal production companies and food canteens which cannot afford increasing price of gas and other technology driven sources of fuel are very common. On the basis of these, there is need to assess sustainable usage of wood ash as soil amendments in order to increase crop production among local farmers. Hence, this study evaluated effects of wood ash liquid fertilizer as soil amendment on agronomic and physiological parameters of *C. annum*.

## **Materials and Methods**

### **Study Area**

The study was conducted in the green house of Department of Botany, Lagos State University, Ojo campus. The geographical references of latitude and longitude of the study area are 6.4668. N6° 28'0.760" and 3.19917 E3°11'57.006" respectively.

### **Sources of Seeds**

Seeds of *C. annum* were collected from Abule, Ojoo, along Federal University of Agriculture Alabata, Abeokuta, Ogun State, Nigeria. The seeds were identified at Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The voucher number of the seed is FHI-51380.

### **Soil collection**

Top soil was collected from areas of the botanical garden of Department of Botany, Lagos State University, Ojo campus. From 500 m apart, top soil was collected at the botanical garden using soil probe as described by Ojewumi et al., (2022). The soil samples were sterilized using the autoclave at 132 °C for 30 minutes.

### **Preparation for Planting**

The soil was poured into twenty five perforated planting buckets. Both the planting buckets and soil weighed 10kg. The perforation allowed drainage and soil aeration without reducing the soil quantity and quality.

### **Nursery preparation**

Seedlings of *C. annum* were raised in the green house of Department of Botany, Lagos State University, Ojo, Lagos for three weeks. The seedlings were randomly transplanted into the planting buckets when they were 14.5 cm high. The seedlings were thereafter watered for one week to ensure acclimatization of the seedling to the planting buckets.

### Experimental design

The planting buckets with one seedling each were arranged in a completely randomized design with five replicates. Also, temperature of the green house was 28 °C in the day and 21 °C in the night while relative humidity ranged from 65–75%.

### Preparation and application of wood ash liquid fertilizer.

Procedure of Ojewumi and Oyetunji,(2022) was modified and used to determine wood ash liquid fertilizer used for this study. Wood ash was obtained from small scale industries in Ojo, Lagos and thoroughly sieved to remove pebbles, then 1000 g of the sieved wood ash was weighed, diluted with 2L of water, mixed thoroughly and filtered. The residue was discarded while the filtrate(wood ash liquid) was used to constitute different level (250 mL liquid wood ash+750 mL water, 500 mL liquid wood ash +500 mL water, 750 mL liquid wood ash +250 mL water, and 1000 mL liquid wood ash) of wood ash liquid fertilizer (filtrates).. Distilled water served as control.

The planting buckets containing one seedling each were divided into five (5) groups and each experimental unit was exogenously applied with one combination of the wood ash liquid fertilizer produced. Seedlings in each group were applied with 50 mL of wood ash liquid fertilizer of each of the combinations prepared above.

### Data collection

Parameters such as plant height, number of leaf, leaf breadth and leaf area were measured. Also, Stem girth was measured using a venire caliper according to the methods of Kadiri (2014). Fresh weight of root and shoot were recorded using sensitive weighing scale. Leaf breadth of controlled and treated plants were measured according to methods of Kadiri (1999) using a thread and a meter rule calibrated in centimeter.

### Determination of total leaf area, specific leaf area and leaf area index of pepper leaves.

A portable Leaf Area Meter (LI-3000 C) manufactured by DMP Ltd was used to determine the leaf area of the seedlings (Ojewumi et al., 2022). Using the leaf area values and weight of leaves, physiological parameters such as specific leaf area (SLA) and leaf area index (LAI) were computed according to methods described by Alireza et al., (2012) as shown below

$$\text{Specific leaf area (SLA)} = \frac{\text{Leaf area(LA)}}{\text{Corresponding weight of leaf(WL)}} \dots\dots\dots (1),$$

where LA = Leaf Area; W<sub>L</sub> = Corresponding weight of leaf

$$\text{Leaf Area Index (LAI)} = \frac{\text{Leaf area(LA)}}{\text{Area of litter fall}} \dots\dots\dots (2).$$

### Determination of net assimilation rate and relative growth rate

The mean values of fresh weights, leaf areas and times of harvest parameters from 5 replicates were used to calculate the following components of growth analysis as described by Badr et al.(1998).

### Relative Growth Rate (RGR), Assimilation Rate (NGR) and Leaf Area Ratio (LAR).

#### Relative Growth Rate (RGR)

Relative growth rate (RGR) was calculated from the measured values of fresh weight of plants (W<sub>1</sub> and W<sub>2</sub>) at time t<sub>1</sub> and t<sub>2</sub> using the mathematical relationship below.

$$\text{RGR} = \frac{\text{Loge}W_2 - \text{Loge}W_1}{t_2 - t_1} \dots\dots\dots (3)$$

### Net Assimilation Rate

The Net Assimilation Rate (NAR) was calculated from the measured values of leaf area ( $A_1$  and  $A_2$ ), fresh weight of plants ( $W_1$  and  $W_2$ ) and time ( $t_1$  and  $t_2$ ).using the relationship below.

$$\text{NAR} = \frac{W_2 - W_1}{A_2 - A_1} \cdot \frac{\text{Loge}A_2 - \text{Loge}A_1}{t_2 - t_1} \dots\dots\dots (4)$$

The Leaf Area Ratio (LAR) was also calculated from the measured values of leaf area ( $A_1$  and  $A_2$ ), fresh weight of plants ( $W_1$  and  $W_2$ ) and time ( $t_1$  and  $t_2$ ) using the relationship below.

$$\text{LAR} = \frac{W_2 - W_1}{t_2 - t_1} \cdot \frac{\text{Loge}A_2 - \text{Loge}A_1}{W_2 - W_1} \dots\dots\dots (5)$$

### Determination of chlorophyll contents in *Capsicum annum*

One gram each of fresh leaf of treated and control plants were obtained from the harvested *C. annum*. The leaves were crushed separately in a clean mortar with 40 mL of 80% (v/v) acetone. The resulting green liquid was transferred into a Buchner Funnel containing a pad of whatman No. 1 filter paper and filtered. The residual pulp was re-extracted with 20 mL aliquot of 80 % acetone and filtered into the previous filtrate. The optical density of the chlorophyll extract in a 10 mm cell was determined with a spectrophotometer set at 652 nm and the amount of chlorophyll present in the leaf was calculated according to Yang *et al.* (2009) using the formula below:

$$\text{Mg total chlorophyll/g tissue} = \frac{0.025 \times \text{O.D.} \times 1000 \times V}{34.5 \times 1000 \times W} \dots\dots\dots (6)$$

Where:

O.D = Optical density reading of chlorophyll extract at 652nm

V = the final volume of the chlorophyll extract

W = the fresh weight (in grams) of the leaf tissue extracted.

### Statistical analysis

Data obtained we reanalyzed using statistical analysis system (SAS, 2013). Means were calculated using one way analysis of variance and separated using Duncan's Multiple Range Test(DMRT) at  $P < 0.05$ .

## Results and Discussion

Table 1 showed that the soil used was acidic (pH; 4.3), organic carbon content was 15.4g kg with total nitrogen (1.3g kg<sup>-1</sup>) and available phosphorus of 19.7g kg<sup>-1</sup>. The textural properties of the soil showed that the soil is sandy loam constituting 830g kg<sup>-1</sup> sandy, 39 g kg<sup>-1</sup>,clay and 131g kg<sup>-1</sup>silt.

The nutritional elements compositions of the soil in terms of potassium, calcium, sodium and magnesium were relatively low. Figure 1 showed that the nutrients and physicochemical contents of wood ash liquid fertilizer were relatively higher than that reported in the soil.

The morphological parameters such as plant height of pepper as influenced by wood ash liquid fertilizer (WALF) application is represented in table 2. It was observed that there was no significant

variation ( $p>0.5$ ) in the height of the pepper treated with different combinations of the WALF at 2 weeks after treatment (WAT). The treatments had significant increase on the parameter treated with combination 500 mL WALF + 500 mL Water) at 4WAT (17.80 cm) and 6 WAT (19.62 cm) compared with other treatments. Similar observation was noticed on number of leaf, leaf length, stem girth and petiole length of pepper treated using WALF at 2 and 4 WAT. Higher number of leaf (16.60), leaf length (3.58 cm), stem girth (1.12 cm) and petiole length (5.62 cm) were recorded in pepper treated with 500 mL WALF+500 mL Water (Table 3). Similar trend was noticed on the effect of the treatment on the leaf breadth (3.58 cm) of the plant (Tables 4).

### Effects of different concentrations of wood ash liquid fertilizer on physiological parameters of pepper

Chlorophyll ( $13.6 \mu\text{mol m}^{-2}$ ) was significantly higher in the leaves of the pepper treated with 500 mL WALF+ 500 mL Water followed by control ( $10.90 \mu\text{mol m}^{-2}$ ) (Figure 2). Higher net assimilation rate ( $0.80 \text{ gm}^{-2}\text{day}^{-1}$ ), relative growth rate ( $0.78 \text{ mgg}^{-1}\text{day}^{-1}$ ) and leaf area ratio ( $0.08 \text{ m}^2\text{kg}^{-1}$ ) were recorded in the leaves of pepper treated with 500 mL WALF+500 mL Water compared with lower or higher concentration of the WALF (Table 8). Also, the treatments had significant increase on Leaf area ( $78.08 \text{ cm}^2$ ) and Specific leaf area ( $160.56 \text{ m}^2.\text{kg}^{-1}$ ) of the pepper treated with 500 mL WALF+ 500 mL Water at 8WAT. Similar observation was noticed on the effects of the treatments on Leaf area ( $83.56 \text{ cm}^2$ ) and Specific leaf area ( $142.56 \text{ m}^2.\text{kg}^{-1}$ ) of the pepper at 10 WAT (Table 9).

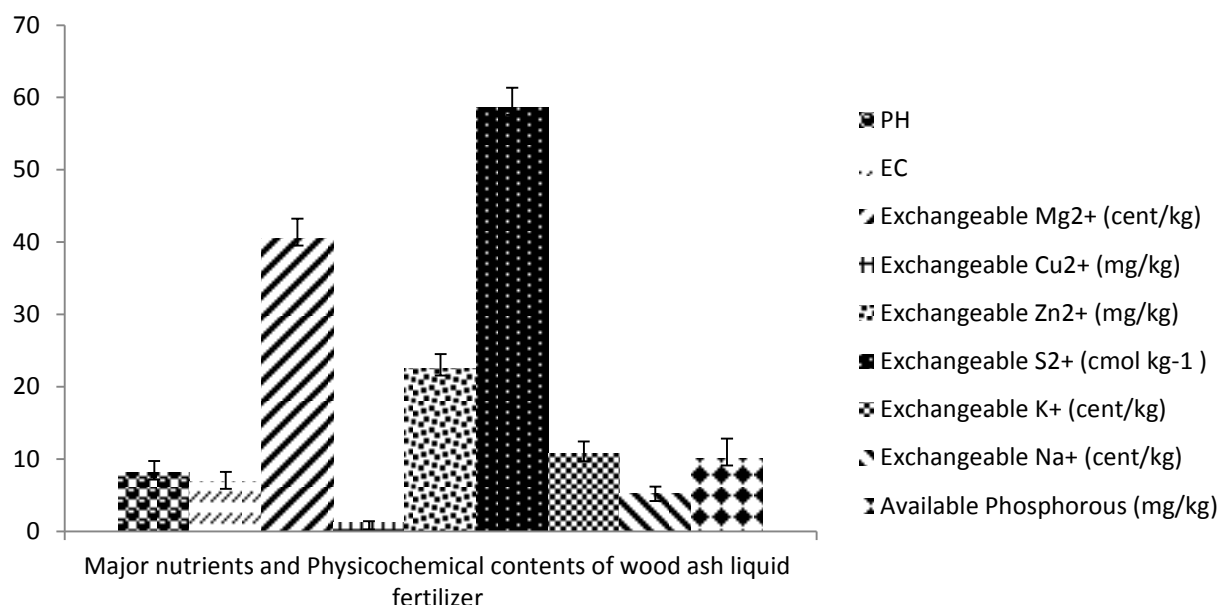
**Table 1.** The physical and chemical properties of the soil used for the experiment

| Parameters                                       | Quantities |
|--------------------------------------------------|------------|
| pH (H <sub>2</sub> O) (1.1)                      | 4.3±1.23   |
| Organic Carbon (g kg <sup>-1</sup> )             | 15.4±1.74  |
| Total N (g kg <sup>-1</sup> )                    | 1.3±0.96   |
| Available P (mg kg <sup>-1</sup> )               | 19.7±1.56  |
| Particle size distribution (g kg <sup>-1</sup> ) |            |
| Sand                                             | 830±2.85   |
| Clay                                             | 39±1.45    |
| Silt                                             | 131±2.95   |
| Textural class                                   | Sandy Loam |
| Exchangeable Bases (cmol kg <sup>-1</sup> )      |            |
| Potassium                                        | 0.33±0.02  |
| Calcium                                          | 6.34±1.45  |
| Sodium                                           | 0.50±0.04  |
| Magnesium                                        | 0.61±0.03  |

**Table 2.** Effects of varying concentrations of wood ash liquid fertilizer on height of pepper

| Treatments (mL)          | Weeks after treatment   |                          |                          |
|--------------------------|-------------------------|--------------------------|--------------------------|
|                          | 2                       | 4                        | 6                        |
| 250 WALF +750 Water      | 13.76±0.66 <sup>a</sup> | 14.92±0.66 <sup>a</sup>  | 14.96±0.72 <sup>ab</sup> |
| 500 WALF +500 Water      | 13.52±1.25 <sup>a</sup> | 17.80±1.86 <sup>a</sup>  | 19.62±0.56 <sup>a</sup>  |
| 750 WALF +250            | 13.08±0.25 <sup>a</sup> | 6.04±1.00 <sup>b</sup>   | 15.88±1.23 <sup>b</sup>  |
| 1000 WALF                | 13.64±0.67 <sup>a</sup> | 14.52±1.00 <sup>ab</sup> | 12.04±0.73 <sup>c</sup>  |
| Control (Ordinary water) | 15.64±1.40 <sup>a</sup> | 16.68±1.63 <sup>a</sup>  | 18.82±0.64 <sup>a</sup>  |

Means  $\pm$  standard error followed by different superscripts in the same columns are significantly deferent at  $p < 0.05$  according to Duncan's Multiple Range Test (DMRT) WALF=Wood ash liquid fertilizer



**Figure 1.** Exchangeable nutrients and physicochemical contents of wood ash liquid fertilizer

**Table 3.** Effects of varying concentrations of wood ash liquid fertilizer on number of leaf of pepper

| Treatments (mL )         | Weeks after treatment         |                               |                               |
|--------------------------|-------------------------------|-------------------------------|-------------------------------|
|                          | 2                             | 4                             | 6                             |
| 250 WALF +750 Water      | 7.60 $\pm$ 1.02 <sup>a</sup>  | 11.00 $\pm$ 0.70 <sup>a</sup> | 11.20 $\pm$ 0.86 <sup>b</sup> |
| 500 WALF +500 Water      | 9.00 $\pm$ 1.26 <sup>a</sup>  | 12.00 $\pm$ 3.42 <sup>a</sup> | 16.60 $\pm$ 0.50 <sup>a</sup> |
| 750 WALF +250            | 10.00 $\pm$ 0.83 <sup>a</sup> | 13.80 $\pm$ 1.15 <sup>a</sup> | 12.20 $\pm$ 0.58 <sup>b</sup> |
| 1000 WALF                | 7.60 $\pm$ 1.16 <sup>a</sup>  | 10.20 $\pm$ 1.59 <sup>a</sup> | 10.60 $\pm$ 1.60 <sup>b</sup> |
| Control (Ordinary water) | 7.60 $\pm$ 1.53 <sup>a</sup>  | 12.80 $\pm$ 1.2 <sup>a</sup>  | 12.40 $\pm$ 1.36 <sup>b</sup> |

Means  $\pm$  standard error followed by different superscripts in the same columns are significantly deferent at  $p < 0.05$  according to Duncan's Multiple Range Test (DMRT) WALF=Wood ash liquid fertilizer.

**Table 4.** Effects of varying concentrations of wood ash fertilizer on leaf length of pepper

| Treatments (mL )         | Weeks after treatment        |                              |                               |
|--------------------------|------------------------------|------------------------------|-------------------------------|
|                          | 2                            | 4                            | 6                             |
| 250 WALF +750 Water      | 4.16 $\pm$ 0.70 <sup>a</sup> | 2.74 $\pm$ 0.16 <sup>a</sup> | 2.00 $\pm$ 0.16 <sup>b</sup>  |
| 500 WALF +500 Water      | 4.10 $\pm$ 1.07 <sup>a</sup> | 2.86 $\pm$ 0.42 <sup>a</sup> | 3.58 $\pm$ 0.32 <sup>a</sup>  |
| 750 WALF +250 Water      | 3.66 $\pm$ 0.50 <sup>a</sup> | 2.96 $\pm$ 0.32 <sup>a</sup> | 2.20 $\pm$ 1.20 <sup>ab</sup> |
| 1000 WALF                | 3.46 $\pm$ 0.50 <sup>a</sup> | 2.46 $\pm$ 0.38 <sup>a</sup> | 2.32 $\pm$ 0.40 <sup>ab</sup> |
| Control (Ordinary water) | 2.96 $\pm$ 0.31 <sup>a</sup> | 2.88 $\pm$ 0.26 <sup>a</sup> | 2.50 $\pm$ 0.40 <sup>ab</sup> |

Means  $\pm$  standard error followed by different superscripts in the same columns are significantly deferent at  $p < 0.05$  according to Duncan's Multiple Range Test (DMRT) WALF=Wood ash liquid fertilizer.



**Table 5.** Effects of varying concentrations of wood ash liquid fertilizer on leaf breadth of pepper

| Treatments (mL)          | Weeks after treatment  |                        |                         |
|--------------------------|------------------------|------------------------|-------------------------|
|                          | 2                      | 4                      | 6                       |
| 250 WALF +750 Water      | 1.62±0.20 <sup>a</sup> | 2.00±0.20 <sup>a</sup> | 2.00±0.20 <sup>ab</sup> |
| 500 WALF +500 Water      | 2.02±0.50 <sup>a</sup> | 2.60±0.33 <sup>a</sup> | 3.58±0.32 <sup>a</sup>  |
| 750 WALF +250 Water      | 1.66±0.30 <sup>a</sup> | 2.16±0.40 <sup>a</sup> | 2.50±0.40 <sup>ab</sup> |
| 1000 WALF                | 1.46±0.23 <sup>a</sup> | 2.38±0.40 <sup>a</sup> | 2.32±0.40 <sup>ab</sup> |
| Control (Ordinary water) | 1.36±0.30 <sup>a</sup> | 1.80±0.70 <sup>a</sup> | 2.20±1.22 <sup>ab</sup> |

Means ± standard error followed by different superscripts in the same columns are significantly deferent at  $p < 0.05$  according to Duncan's Multiple Range Test (DMRT) WALF=Wood ash liquid fertilizer.

**Table 6.** Effects of varying concentrations of wood ash liquid fertilizer on stem girth of pepper

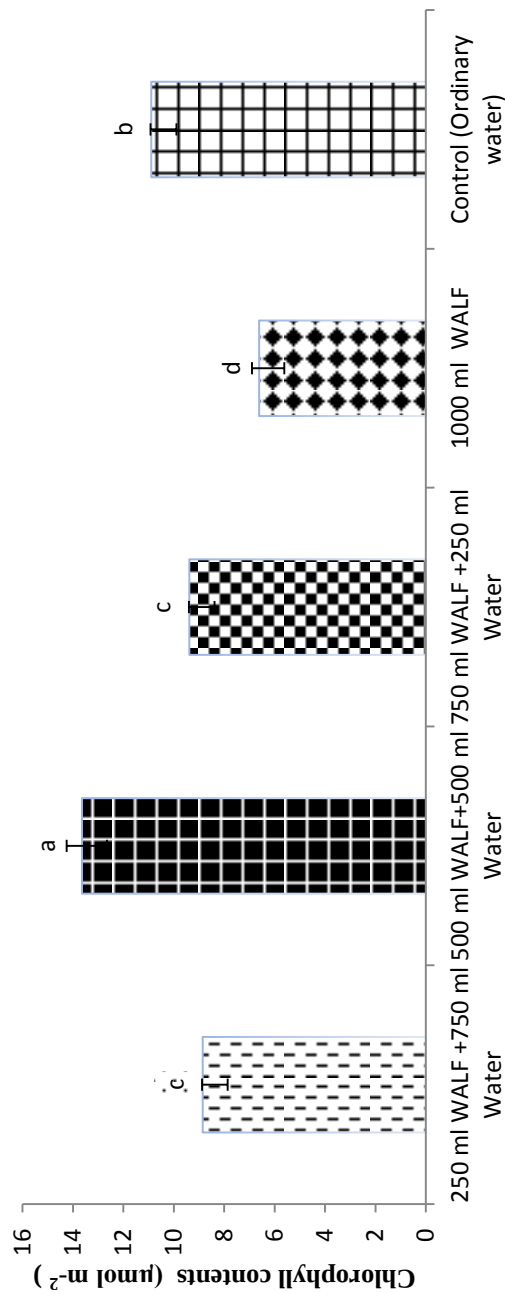
| Treatments (mL )         | Weeks after treatment   |                         |                         |
|--------------------------|-------------------------|-------------------------|-------------------------|
|                          | 2 WAT                   | 4WAT                    | 6WAT                    |
| 250 WALF +750 Water      | 0.62±0.10 <sup>a</sup>  | 0.80±0.70 <sup>ab</sup> | 0.80±0.10 <sup>b</sup>  |
| 500 WALF +500 Water      | 0.78±0.40 <sup>b</sup>  | 0.90±0.13 <sup>a</sup>  | 1.12±0.10 <sup>a</sup>  |
| 750 WALF +250 Water      | 0.64±0.1 <sup>ab</sup>  | 0.78±0.73 <sup>ab</sup> | 0.68±0.11 <sup>b</sup>  |
| 1000 WALF                | 0.58±0.03 <sup>a</sup>  | 0.68±0.10 <sup>c</sup>  | 0.72±0.04 <sup>b</sup>  |
| Control (Ordinary water) | 0.72±0.05 <sup>ab</sup> | 0.78±0.80 <sup>ab</sup> | 0.96±0.14 <sup>ab</sup> |

Means ± standard error followed by different superscripts in the same columns are significantly deferent at  $p < 0.05$  according to Duncan's Multiple Range Test (DMRT) WALF=Wood ash liquid fertilizer,

**Table 7.** Effect of different concentrations of wood ash liquid fertilizer on petiole length of pepper

| Treatments (mL )         | Weeks after treatment   |                        |                         |
|--------------------------|-------------------------|------------------------|-------------------------|
|                          | 2                       | 4                      | 6                       |
| 250 WALF +750 Water      | 2.36±0.40 <sup>a</sup>  | 3.48±0.8 <sup>a</sup>  | 4.54±0.81 <sup>b</sup>  |
| 500 WALF +500 Water      | 1.48±0.33 <sup>ab</sup> | 4.96±1.22 <sup>a</sup> | 5.62±1.34 <sup>a</sup>  |
| 750 WALF +250 Water      | 1.24±0.20 <sup>b</sup>  | 4.00±1.10 <sup>a</sup> | 4.70±0.98 <sup>b</sup>  |
| 1000 WALF                | 1.60±0.31 <sup>ab</sup> | 3.28±0.80 <sup>a</sup> | 3.30±0.80 <sup>a</sup>  |
| Control (Ordinary water) | 0.88±0.20 <sup>b</sup>  | 3.62±0.93 <sup>a</sup> | 5.00±0.42 <sup>ab</sup> |

Means ± standard error followed by different superscripts in the same columns are significantly deferent at  $p < 0.05$  according to Duncan's Multiple Range Test (DMRT)WALF=Wood ash liquid fertilizer,



**Figure 2.** Effects of different concentrations of wood ash liquid fertilizer on chlorophyll contents of pepper. WALF=Wood ash liquid fertilizer.

**Table 8.** Effect of different concentrations of wood ash liquid fertilizer on Growth parameters and some Physiological Parameters of Pepper leaves

| Treatments (mL )         | 8WAT                    |                           | 10WAT                   |                          | NAR(gm <sup>-2</sup> day <sup>-1</sup> ) | RGR(Mgg <sup>-1</sup> day <sup>-1</sup> ) | LAR(m <sup>2</sup> kg <sup>-1</sup> ) |
|--------------------------|-------------------------|---------------------------|-------------------------|--------------------------|------------------------------------------|-------------------------------------------|---------------------------------------|
|                          | Leaf area(cm)           | Specific leaf             | Leaf area(cm)           | Specific leaf            |                                          |                                           |                                       |
| 250 WALF +750 Water      | 60.12±5.39 <sup>b</sup> | 116.70±2.79 <sup>b</sup>  | 63.48±1.48 <sup>b</sup> | 73.12±1.47 <sup>d</sup>  | 0.58±0.31 <sup>ab</sup>                  | 0.20±0.10 <sup>b</sup>                    | 0.02±0.01 <sup>b</sup>                |
| 500 WALF +500 Water      | 78.08±4.46 <sup>a</sup> | 160.56±3.40 <sup>a</sup>  | 83.56±1.93 <sup>a</sup> | 142.56±2.59 <sup>a</sup> | 0.80±0.37 <sup>a</sup>                   | 0.78±0.49 <sup>b</sup>                    | 0.08±0.02 <sup>a</sup>                |
| 750 WALF +250 Water      | 54.76±0.97 <sup>c</sup> | 120.86±4.36 <sup>a</sup>  | 56.24±2.64 <sup>c</sup> | 103.60±1.15 <sup>c</sup> | 0.66±0.54 <sup>ab</sup>                  | 0.19±0.10 <sup>b</sup>                    | 0.02±0.01 <sup>b</sup>                |
| 1000 WALF                | 51.34±1.44 <sup>c</sup> | 84.45±1.27 <sup>a</sup>   | 36.56±7.23 <sup>d</sup> | 70.96±2.08 <sup>d</sup>  | 0.44±0.30 <sup>b</sup>                   | 0.21±0.05 <sup>b</sup>                    | 0.02±0.01 <sup>b</sup>                |
| Control (Ordinary water) | 54.84±4.73 <sup>c</sup> | 100.08±2.23 <sup>ac</sup> | 54.86±6.45 <sup>c</sup> | 112.48±5.29 <sup>b</sup> | 0.20±0.00 <sup>a</sup>                   | 0.78±0.04 <sup>a</sup>                    | 0.04±0.01 <sup>ab</sup>               |

Means ± standard error followed by different superscripts in the same columns are significantly deferent at  $p < 0.05$  according to Duncan's Multiple Range Test (DMRT) WALF=Wood ash liquid fertilizer, WAT= Weeks after treatment

## Discussion

Sustainable usage of waste products such as wood ash generated by small scale industries is an innovative approach towards improvement of crop production and reduction of food insecurity most especially in developing countries such as Nigeria. Analysis of soil where this study was carried out indicates that textural properties of the soil can hold moisture or water needed for cultivation of crops such as pepper yet the mineral composition may not be adequate to meet nutritional requirement for cultivation of most vegetables (Olugbemi, 2019). This observation therefore could be regarded as reasonable justification for enhancement of the soil using plant based organic material such as wood ash (Olugbemi, 2019).

These findings also showed influence of wood ash on soil enrichment for proper growth, development of physiological parameters and yield of the pepper. Better performance of agronomic characters such as height, number of leaf and leaf area recorded in pepper treated with combination 500 mL WALF + 500 mL Water may indicate that the combination contained higher nutritional contents required for growth and development of pepper. This is in agreement with submission of Moure et al.(2020) who opined that two different blending combinations of food waste and agro-industrial waste digestates, and wood ashes released nutrients to plants than when each of them was used singly. Mbah et al. (2009) and Nweke et al. (2017) also reported that application of combinations of poultry manure improved better maize growth and yield.

In a similar order, higher leaf length, leaf breadth, stem girth and petiole length recorded in the pepper treated with 500 mL WALF + 500 mL Water at 6 WAT compared with other treatments may be due to the presence of appreciable amount of exchangeable minerals recorded in the filtrates of the ash as a required factor for tissue development of the vegetable. Also, an increase in number of leaf per plant observed in the pepper as affected by application of WALF is in agreement with submissions of Makinde (2007) and Jenni et al. (2008) who posited that synergy of combination of poultry manure and cow dung improved vegetative growth of crops. This observation infers that nutrient depleted soil could be enriched using ash to introduce nutrients to the soil thereby influencing the morphological characters and other functional units of plants (Saarsalmi et al., 2001).

Furthermore, 1000 mL WALF reduced morphological and physiological characters of the pepper. The reduction may indicate that the concentration of the treatment was higher than maximum requirement of the vegetable which eventually retarded or inhibited general development of the vegetables. Also, application of 50 mL of 1000 mL WALF might have interacted negatively with tubular hair as conducting vessels of the vegetable.

In addition, leaves of the pepper treated with 1000 mL WALF became yellow, wilted and eventually got defoliated. This may be due to the degradation actions of the treatment on chlorophyll content and undue secretion and self-digesting enzymes by parenchymatous cells of the pepper on middle lamella which eventually resulted into detachment of the leaves.

The treatment at that concentration may on the other hand be used as herbicides to control weeds. High chlorophyll content recorded in the leaves of pepper treated with 500 mL WALF+500 mL Water could suggest that the combination formed a nutritional synergy which enhanced chlorophyll and biochemical pigments formation. This assertion is in agreement with submission of Mohamed et al. (2008) who opined that that chlorophyll content and photosynthates are biochemical molecules formed as a result of plant metabolic activities. Lowest quantity of chlorophyll recorded in the pepper treated with 1000 mL of the filtrates might have disintegrated the chlorophyll content of the plants as reflected in the yellowing and eventually defoliation of the leaves due to its high concentration.

In the same trend, higher leaf area and net assimilation rate recorded in the pepper treated with the combination could be a measure of size of assimilatory system, accumulation and partitioning of photosynthates and economic relevance of the plant (Fanuel and Gifole, 2013). Studies of Subedi and Ma, (2005) and Rasheedat et al. (2017) had it that leaf area is one of the major components influencing plant productivity, dry matter production and grain yield of plant. Also, significant effect of application of 500 mL WALF + 500mL Water observed on leaf area, specific leaf area, net assimilation rate and relative growth rate of pepper could be a magnitude of total leaf area values which were higher in pepper treated using the treatment. The order of size of total leaf area values recorded in pepper treated with the combination could be responsible for high number of leaves recorded on the vegetable treated with the combination.

## Conclusion

This study revealed that although wood ash liquid fertilizer has positive effects on growth and physiological attributes of the pepper, however, WALF at combination 500 mL WALF + 500 mL Water enhanced maximum growth and better physiological performance of the plant. In addition, 1000 Wood ash liquid fertilizer retarded morphological characters and physiological attributes of the pepper, therefore, combination of 500 mL wood ash liquid fertilizer (WALF) + 500 mL Water was regarded as optimal ratio (1:1) of the wood ash for pepper production.

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## Seed Priming Improves Growth and Yield of Moisture-Stressed Rice at Tillering Stage



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### Abstract

Moisture stress is among the major banes against production of rice at self-sufficient level. Different growth stages of rice are affected differently by moisture stress. Therefore, we conducted a study to determine the potential of seed priming in alleviating moisture stress in rice at tillering stage. The priming treatments used were 100mM calcium chloride dehydrate, 40% (w/v) polyethylene glycol (PEG) 6000 and 100ppm kinetin. Pre-germination which is the farmers' practice was used as the control. We assessed the plants on number of tillers, productive tillers, tillering efficiency and sterility, number of spikelets, filled spikelets, 100 grain weight, grain yield, harvest index, chlorophyll and proline concentration, net photosynthesis, stomatal conductance, inter cellular carbon dioxide and transpiration rate. We found that one day priming with 100ppm kinetin conferred better tolerance against moisture stress and produced higher grain yield than the rest treatments at tillering stage. The outcome of this research is that by using one-day priming with 100ppm kinetin for production of MR219, we can save water at tillering stage and use the saved water for nurturing the plants at booting stage which is ultra-sensitive to water stress.

**Keywords:** seed priming, moisture stress, growth stages, tillering stage, gas exchange characteristics, rice.

### Introduction

The occurrence of water stress for varying intensity and duration could be at different growth stages and such can adversely affect growth, development and yield of plants. When water stress occurs at the vegetative stage, it results in reduction in the number of tillers per hill. This effect seems to be mild compared to imposition of the same stress at reproductive stages. Nevertheless, response to water stress is varietal dependent. This is because some varieties are severely affected at the vegetative stage whereas others are affected at the reproductive stage.

Leaf is the most sensitive organ to environmental stresses (Nevo et al., 2000). Therefore, the effect of water stress is majorly noticed in it more than any other part of the plant. The anatomical

and visual changes found in the leaves during moisture stress period consequently change the diffusion component of carbon dioxide conductance from sub-stomatal area to carboxylation sites and, therefore, contribute to photosynthesis maintenance despite low rate of stomatal conductance (Evans and Loreto, 2000). Mesophyll and stomatal conductance are potentially responsible for reduction in photosynthetic rate when plants are subjected to moisture stress. This implies that the main photosynthetic limitations are diffusional limitations caused by alterations found in the leaf stomata (Loreto et al., 2003)

Chlorophyll which is a light-harvesting pigment in plant is affected by moisture stress. Moreover, changes in chlorophyll *a* and *b* content are noticeable in drought stressed plants (Farooq et al., 2009). Alteration in chlorophyll components and destruction of photosynthetic apparatus and consequent changes in chlorophyll content can reduce net photosynthesis during severe moisture stress (IturbeOrmaetxe et al., 1998). This is because moisture stress decreases chlorophyll contents of the leaves (Ommen et al., 1999). In the same vein, moisture stress results in decrease in chlorophyll *a*, *b* as well as total chlorophyll contents (Manivannan et al., 2007). The reduction in chlorophyll contents could be attributed to damage caused by reactive oxygen species (ROS) to the chloroplast (Smirnoff, 1995) because drought stress leads to imbalanced production of ROS.

Plants do engage in production of some chemicals (osmolytes) to safeguard them from the destructive effects of moisture stress. Among the produced osmolytes is proline which is believed to be commonly produced by plants under stress like drought, high temperature and starvation (Sairam et al., 2002). Accumulation of proline under moisture stress has been established in pea (Alexieva et al., 2001). Metabolism of proline in relation to osmotic stress has been studied (Verbruggen and Hermans, 2008). Proline accumulation in plants is an indication of environmental stress and could be used as an adaptive response of plants under stress (Maggio et al., 2002).

The challenging problem of moisture stress in rice production calls for a better way out that is simple, effective and adoptable. Among the various strategies that could be used in alleviating moisture stress, seed priming is the physiological approach that is highly effective, with low cost and risk, simple and adoptable (Wahid and Shabbir, 2005). The resulting plants from seed priming could tolerate future stress because their seeds have been subjected to certain degree of stress during priming treatment (Bruce et al., 2007).

The selected treatments from Kareem et al. (2020) which were a two-day osmo-priming and one-day hormonal priming were tested against pre-germination treatment which is the common practice in the granary areas of the country. The objective of this research was, therefore, to determine the potential of seed priming in alleviating moisture stress in rice at tillering stage.

## **Materials and Methods**

### **Experimental Site**

An experiment to assess the efficacy of rice seed priming in overcoming the problem of incessant water shortage during the subsidiary production season production or unexpected delay in rain to start and maintain rice production on the field was conducted in Rice Research Centre glass house of Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia (3° 02' N, 101° 42' E; elevation 31 m). The average monthly maximum and minimum temperatures were 33.5 °C and 21.5 °C respectively while the relative humidity is 92.5 %. The sunshine hour is 6.6 h /day while the average rainfall and evaporation are 9.8 mm/day and 4.6 mm/day respectively.

## Plant Materials and Experimental Design

The seeds used in this experiment were collected from the gene bank of the faculty of agriculture, UPM. The rice variety researched on was MR219. The priming treatments used were 100mM calcium chloride dehydrate, 40% (w/v) polyethylene glycol (PEG)6000 and 100ppm kinetin. Pre-germination which is the farmers' practice was used as the control. The experiment was laid out in randomized complete block design (RCBD) with three replications.

## Agronomic and cultural practices

23kg Kelantan soil series was filled into each of the experimental pot (experimental unit) with diameter 31.5cm and height 36.5cm. The soil was puddled to create an impervious layer which prevents uncontrolled percolation of water and leaching of the nutrient. The water level was then reduced to the barest minimum to pave way for seed-soil-water contact that led to seed germination. Ten seeds were planted per pot. The germinating seeds were irrigated regularly but high level of standing water was prevented to avoid flooding injury. The seedlings were finally reduced to four plants per pot and weed-free till the end of the experiments. The plants were raised under anaerobic condition till the time of stress imposition. After the end of stress period, the initial anaerobic condition was restored and maintained till harvesting.

## Stress treatment

At tillering stage (51 days after planting), plants were subjected to a 19-day moisture stress stage). The pots were drained and irrigation was stopped for nineteen days to test their tolerance to water deficit. Irrigation was restored when soil moisture content was 7%.

## Data Collection

### Plant Phenology and Yield Data

Days to flowering was counted from the time of seeding to the appearance of panicle. Number of filled and unfilled grains or spikelets, weight of 100 seeds, final yield per treatment as well as harvest index were all recorded after harvesting. Harvest index was calculated as follows:

$$\text{Harvest Index} = \frac{\text{weight of Economic yield}}{\text{weight of Biological yield}} \times 100$$

### Physiological and Biochemical Data

Determination of leaf chlorophyll content was done following the procedure in Coombs et al. (1985).

### Proline Determination

Leaf proline content was estimated colorimetrically using the acid ninhydrin method of Bates et al. (1973).

### Determination of Leaf Gas Exchange Characteristics

Data on net photosynthetic rate ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ ), intercellular carbon dioxide ( $\mu\text{mol CO}_2\text{m}^{-1}$ ), stomatal conductance ( $\mu\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$ ) and transpiration rate ( $\text{mmol m}^{-2}\text{s}^{-1}$ ) were taken with a closed infra-red gas analyzer LICOR 6400 Portable Photosynthesis System (IRGA, Licor Inc., Lincoln, NE, USA) following the procedure of Ibrahim and Jaafar of 2012.

## Data Analysis

All the collected data collected in experiments were analysed using analysis of variance (ANOVA) by following RCBD procedure. Significant means were then separated using the least significant difference (LSD).

## Results and Discussions

### Effect of seed priming on number of tillers of moisture-stressed rice at tillering stage

The highest number of tillers was recorded from plants resulting from pre-germination followed the plants from calcium chloride priming while plants from kinetin and PEG priming had the lowest number of tillers (Table 1). The vigour gained by pre-germinated seeds was advantageous to the succeeding plants and paved the way for having enhanced number of tillers than the counterpart priming media. Moreover, earliness in the beginning of life of pre-germinated seeds was a strong force necessitating having higher number of tillers than the priming treatments that started pretty late. Furthermore, imposition of stress at tillering stage did not give full time opportunity to the plants from priming treatments to express their full growth potential.

### Effect of seed priming on number of productive tillers and tillering efficiency of moisture- stressed rice at tillering stage

The highest number of productive tillers was from plants resulting from kinetin priming followed by plants from calcium chloride priming while the lowest number of productive tillers was from PEG priming (Table 1). The highest percentage of tiller efficiency was from kinetin priming followed by calcium chloride priming while the lowest efficiency was recorded from pre-germination treatment (the control) (Table 1). Productive tillers are pre-requisites for production of higher grain yield provided panicle blanking is prevented through ineffective assimilate partitioning. This performance of kinetin here has given the plants resulting from its treatment to have higher grain yield. Better performance of kinetin priming in tiller productivity was further strengthened by high tiller efficiency and low sterility percentage. Contrary to this, the control as well as the rest priming media had low tiller efficiency and higher sterility percentage.

**Table 1.** *Effect of seed priming on number of tillers of moisture-stressed rice at tillering stage*

| Treatments        | Number of tillers | Number of Effective Tillers | Tiller Efficiency (%) |
|-------------------|-------------------|-----------------------------|-----------------------|
| CaCl <sub>2</sub> | 43.00b            | 37.00b                      | 77.63b                |
| PEG               | 42.00b            | 18.00d                      | 56.24c                |
| Kinetin           | 42.00b            | 40.00a                      | 89.11a                |
| Pre-germination   | 59.00a            | 32.00c                      | 49.21d                |

*Means with the same letters in a column are not significantly different from each other*

### Effect of seed priming on plant height of moisture-stressed rice at tillering stage

The tallest plants were those from PEG followed by those from kinetin while the shortest ones were those from the control (pre-germination treatment) (Table 2). It could be said that meristematic activities in the plants resulting from priming treatments were more enhanced than that of pre-germination (Werner *et al.*, 2001) since growth division that leads to higher height is the prerogative of the meristematic cells. Moreover, it might still be said that PEG priming aided

physiological activities during plant morphogenesis (Igari et al., 2008). Similarly, absorption of nutrients and water by the roots for better vegetative growth might have been enhanced by the priming chemicals. The degree of enhancement in height was proportional to the level of enhancement in height gain. The superiority of PEG priming still revolves round the fact than its level of stress imposition on the primed seeds during treatment was more than any other priming counterpart. So, when the resulting plants were subjected to real environmental stress similar to the simulated stress by the priming chemicals, the plants thrived with ease the way a vaccinated child scale through of a disease epidemic.

**Table 2.** *Effect of seed priming on plant height and number of spikelets of moisture- stressed rice at tillering stage*

| Treatments        | Plant Height(cm) | Number of Spikelets per Panicle |
|-------------------|------------------|---------------------------------|
| CaCl <sub>2</sub> | 86.10b           | 112.00a                         |
| PEG               | 90.00a           | 107.00a                         |
| Kinetin           | 87.30b           | 116.00a                         |
| Pre-germination   | 83.60c           | 103.00a                         |

*Means with the same letters in a column are not significantly different from each other*

#### **Effect of seed priming on number of spikelets and number of filled spikelets per panicle of moisture-stressed rice at tillering stage**

The highest number of spikelets was from kinetin priming followed by calcium chloride priming while the lowest number of spikelets was from pre-germination (the control) (Table 2). The number of filled grains per panicle did not follow the same pattern as the spikelet production. So, the highest number of filled spikelets was from calcium chloride priming followed by PEG priming while the lowest number of filled spikelets was from kinetin priming (Table 3). This could be attributed to the fact that having large panicle size through increase in number of spikelets will significantly increase the number of poorly filled grains while most of the grains in the inferior spikelets will become source-limited (Kato, 2004). This might be because poor partitioning and translocation of assimilates from the source leaves and stems during grain filling could not sustain the development and filling of a large number of spikelets (Yang et al., 2002). Moreover, starch synthesis in the endosperm cells of inferior spikelets is poor (Umemoto et al., 1994) and assimilates partitioned to them remain unused.

#### **Effect of seed priming on 100-grain mass of moisture-stressed rice at tillering stage**

The heaviest seeds were from plants resulting from kinetin and PEG priming followed by pre-germination while the lightest seeds were from calcium chloride priming (Table 3). Increase in grain mass observed in this work with kinetin priming could be attributed to better assimilate partitioning by mitigating the effect of moisture stress. The mass of individual seeds produced from kinetin has contributed to the mass of the final grain yield (Table 3). This could have been the result of better assimilate partitioning though that might have very little or no relationship at all with the light saturated-photosynthesis (Murchie et al., 2002) which might have resulted from the fact that photosynthesis is not affected by antisense which suppresses sucrose transporter gene which impairs rice grain filling (Schofield et al., 2002).

**Table 3.** Effect of seed priming on number of filled spikelets, 100-grain mass and grain yield of moisture-stressed rice at tillering stage

| Treatments        | Number of Filled Spikelets | 100-Grain Mass(g) | Grain Yield(g/pot) |
|-------------------|----------------------------|-------------------|--------------------|
| CaCl <sub>2</sub> | 69.00a                     | 3.63b             | 46.41a             |
| PEG               | 65.00a                     | 3.80b             | 46.12a             |
| Kinetin           | 60.00a                     | 3.80a             | 52.44a             |
| Pre-germination   | 61.00a                     | 3.77b             | 46.66a             |

Means with the same letters in a column are not significantly different from each other

#### Effect of seed priming on grain yield of moisture-stressed rice at tillering stage

The highest yield was from kinetin priming followed by pre-germination treatment while PEG priming produced the lowest grain yield (Table 3). The effect of yield reduction resulting from moisture stress was alleviated through kinetin priming because the treatment produced higher grain yield than the rest priming media including pre-germination treatment which was used as a check because it was the farmers' practice (Table 3). This implies that the effect of moisture stress in grain yield reduction could be alleviated through priming especially hormonal priming like kinetin. This might be because the strength gained through priming with kinetin at vegetative stage mitigated the problems of reduction in grain mass and spikelet fertility which have been identified to be the major sources of yield reduction in rice that is stressed at reproductive stage (Ji et al., 2012). Moreover, it could be that kinetin priming reduced the effect of decrease in assimilate translocation to the reproductive organs of the plant (Hsiao and Xu, 2000) which occurs to rice plant under moisture stress. In the same vein, production of higher grain yield with kinetin priming might have resulted from increase in number of actively reproducing plant cells in inflorescence meristems (Leibfried et al., 2005).

#### Effect of seed priming on harvest index (HI) of moisture-stressed rice at tillering stage

The highest value of harvest index was from kinetin priming followed by PEG priming while the lowest value of harvest index was from pre-germination treatment (Table 4). The success of the priming treatments in enhancing effective assimilate partitioning to the filling grains at the reproductive stage might have led to having higher yield and consequently HI. Having higher HI has been established to be the differentiating factor between wild ancestors and cultivated varieties (Gepts, 2004). Environmental factors like temperature (Prasad et al., 2006) and soil conditions which affect the yield are the primary determinants of what the HI will be. Similarly, plant height does affect the HI (Yang and Hwa, 2008) because it determines the plant biomass. Its relationship is indirect and hence, it is not that desired to have too tall plants. The indirect relationship might also be linked to the problem of lodging in tall rice cultivars (Hommaa et al., 2004) which is antithetical to massive assimilate translocation from the vegetative parts to the filling grains in semi-dwarf cultivars (Zou et al., 2003).



**Table 4.** Effect of seed priming on harvest index (HI), net photosynthesis and stomatal conductance of moisture-stressed rice at tillering stage

| Treatments        | Harvest index |                                                                     |                                                                         |
|-------------------|---------------|---------------------------------------------------------------------|-------------------------------------------------------------------------|
|                   | (%)           | Photosynthesis<br>( $\mu\text{molCO}_2\text{m}^{-2}\text{s}^{-1}$ ) | Stomatal conductance<br>( $\text{molH}_2\text{O m}^{-2}\text{s}^{-1}$ ) |
| CaCl <sub>2</sub> | 39.71b        | 0.727b                                                              | 0.025a                                                                  |
| PEG               | 39.79b        | 1.504a                                                              | 0.029a                                                                  |
| Kinetin           | 50.05a        | 0.139c                                                              | 0.028a                                                                  |
| Pre-germination   | 22.54c        | 0.189c                                                              | 0.017b                                                                  |

*Means with the same letters in a column are not significantly different from each other*

#### Effect of seed priming on stomatal conductance of moisture-stressed rice at tillering stage

The peak of stomatal conductance was from PEG priming followed by kinetin priming while the lowest was from the control (pre-germination)(Table 4). The maintenance of stomatal opening which kept the conductance at higher rate might have been because priming treatments (PEG and kinetin) did not allow the plants much abscisic acid (ABA) that is responsible for stomatal closure. This is so because the mechanism of stomatal closure during moisture stress is closely linked with ABA because it is amply produced by plants under stress and is, therefore, called stress hormone (Melcher et al., 2009). The mechanism of its operation is that it increases production of cytosolic  $\text{Ca}^{2+}$  and activates plasma membrane-localized channels (Kohler and Blatt, 2002). Consequently, there is efflux of potassium, depolarization of guard cells, loss of turgor and guard cell volume, high production of hydrogen peroxide and eventual closure of the stomata (Wang et al., 2012).

#### Effect of seed priming on net photosynthesis of moisture-stressed rice at tillering stage

The highest photosynthetic rate was recorded in plants from PEG priming followed by calcium chloride priming while the lowest rate was recorded in plants from kinetin priming (Table 4). The highest photosynthetic rate recorded from PEG priming had its root from the stomatal conductance capacity of the resulting plants from PEG priming (Table 4). This implies that stomatal conductance is directly related to photosynthetic rate as it has been established because it is responsible for exchange of gases. This further establishes the relationship between photosynthesis and stomatal conductance (Flexas et al., 2002).

Higher photosynthetic rate through seed priming under moisture stress is an indication of less limitation in both stomatal and non-stomatal (metabolic) factors. The lower values recorded for kinetin priming indicates that there were both stomatal and non-stomatal (metabolic) limitations for decrease in photosynthetic activities. This photosynthetic activity inhibition or decrease found in kinetin priming in this work might result in production of higher level of reactive oxygen which may consequently cause oxidative damage to lipid, proteins, DNA and photosynthetic apparatus (Xiong et al., 2002). This reactive oxygen species causes lipid peroxidation which its extent is measured by malondialdehyde (MDA) content in the tissues (Wang et al., 2014). In the same vein, reduction in photosynthetic rate under moisture stress is the result of its adverse effect electron transport among photosystems I, II and enzymes of carbon metabolism like ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) as well as those enzymes related to its synthesis (Medrano et al., 2002).

### Effect of seed priming on intercellular carbon dioxide of moisture-stressed rice at tillering stage

The highest volume of intercellular carbon dioxide (CO<sub>2</sub>) was found in the plants resulting from kinetin priming followed by pre-germination treatment while the lowest concentration was found in the plants from PEG priming (Table 5). Leaves from plants originating from kinetin primed seeds had the highest volume of CO<sub>2</sub> despite the fact that both stomatal conductance and net photosynthesis were at the lowest levels in them. This is a different dimension from the earlier established fact that strong correlation exists between stomatal conductance and inter-cellular CO<sub>2</sub> along with net photosynthesis (Flexas et al., 2002). This result might be because the absorbed CO<sub>2</sub> was judiciously used to leave a significant amount in the intercellular space. This is because the stomatal conductance that should have led to higher accumulation of the gas was low and the photosynthesis that consumes it was lower compared to other treatment (Table 5). Our outcome in this work suggests that the amount of CO<sub>2</sub> does not determine the photosynthetic rate as it is believed that reduction in photosynthetic rate is more influenced by intercellular CO<sub>2</sub> than leaf water potential and leaf water content (Galmes et al., 2011).

### Effect of seed priming on transpiration rate of moisture-stressed rice at tillering stage

The best transpiration rate is the lowest under drought stress. Therefore, the best treatment was pre-germination followed by calcium chloride priming while worst treatment was PEG priming (Table 5). The role of pre-germination was also showcased in this experiment because its regulation against water loss was better than the rest of the priming treatments (Table 5). The role of pre-germination in this work is distinct and evident because it had lower transpiration rate. The consequential gain of this phenomenon was realized in the final yield increase as against other priming media with higher transpiration rate and hence more water loss. This conservation of water is attributed to stomatal closure experience at different growth stages when moisture stress was imposed (Table 5).

**Table 5.** Effect of seed priming on intercellular CO<sub>2</sub>, transpiration, proline and chlorophyll content of moisture-stressed rice at tillering stage

| Treatment         | Intercellular CO <sub>2</sub> ( $\mu\text{molCO}_2\text{m}^{-1}$ ) | Transpiration rate ( $\text{mmolH}_2\text{O m}^{-2}\text{s}^{-1}$ ) | Proline Content( $\mu\text{M}$ ) | Total Chlorophyll Content ( $\mu\text{molcm}^{-2}$ ) |
|-------------------|--------------------------------------------------------------------|---------------------------------------------------------------------|----------------------------------|------------------------------------------------------|
| CaCl <sub>2</sub> | 330.21c                                                            | 0.97b                                                               | 30.56c                           | 4.71a                                                |
| PEG               | 288.30d                                                            | 1.34a                                                               | 33.08b                           | 4.95a                                                |
| Kinetin           | 365.67a                                                            | 1.24a                                                               | 29.75c                           | 3.98b                                                |
| Pre-germination   | 359.00b                                                            | 0.71b                                                               | 38.00a                           | 4.01b                                                |

Means with the same letters in a column are not significantly different from each other

### Effect of seed priming on proline content of moisture-stressed rice at tillering stage

The highest concentration of proline was found in plants that resulted from pre-germination treatment followed by plants PEG priming while the lowest concentration was found in plants from

kinetin priming (Table 5). It is now clear that stress leads to proline production as found in this work. The concentration found in pre-germination is an indication that pre-germination can also initiate stress tolerance in rice although the physiological intervention did not result in final yield increase. As for kinetin, its ability to produce higher concentration may be an indication of its potential for stress tolerance in rice. Conversely, the mitigation of moisture stress effect did not result in higher grain yield as first noted when stress was imposed. At any instance, it could be inferred that having correct proportion of osmolyte production can only enhance plant tolerance level of the adverse situation and not the grain production directly. This is because accumulation of osmolytes like proline is only an adaptive measure against environmental stresses (Cattivelli et al., 2008).

### **Effect of seed priming on leaf chlorophyll content of moisture-stressed rice at tillering stage**

The highest chlorophyll content was found in leaves of plants from PEG priming followed by calcium chloride priming while the lowest chlorophyll content was found in the leaves of plants from kinetin priming (Table 5). This result might be because PEG priming prevented damage that could be done to the chloroplast by reactive oxygen species which are normally produced as a result of moisture or other environmental stress (Smirnoff 1995). The assertion that high chlorophyll content will result in higher yield (Sengupta and Majumder, 2009) was disproved by PEG priming treatment. Similarly, the observation that higher chlorophyll content is a pre-requisite for high grain yield in rice (Wankhade and Sanz, 2013) was not supported by our result from PEG priming. This might be because chlorophyll level is directly linked to photosynthesis only. So, when the concentration of chlorophyll is high, photosynthetic rate will be high as found in this experiment (Table 4). Nevertheless, photosynthesis has been linked to dry matter yield alone and not the grain yield. But if assimilate partitioning is effective, then photosynthesis will have larger contribution to the grain yield and harvest index will be appreciably high.

### **Conclusions**

From this work, it was found that the priming treatments were better than pre-germination in most of the parameters measured. However, one day priming with 100ppm kinetin was able to tolerate the imposed moisture stress appreciably better than the rest treatments because it led to production of the highest grain yield. It was, therefore, concluded that one day priming with 100ppm kinetin could be used for production of MR219 when there is drought at the tillering stage in the area where the research was conducted and areas with similar climatic and conditions.

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## **80 Years since the Death of Nikola Pushkarov - Founder of Soil and Earth Science, and Fighter for National Unity**



**Prof. Metodi Teoharov**

*Chairman of the Bulgarian Soil Science Society*

It has been 80 years since the death of the prominent Bulgarian scientist, naturalist, founder of Bulgarian soil science, patriot and fighter for national unity, public figure, national awakener and revolutionary Nikola Pushkarov.

Pushkarov is one of those individuals who do not fit their life and deeds into a certain framework, and with their ideas and struggles they remain an example to be followed for centuries. In societies filled with injustice and spiritual emptiness, they remain misunderstood or humiliated during their lifetime. In the past and now, the examples are many. I take the liberty of writing words of praise and recognition for the man whose life and work have been strewn with unprecedented difficulties and fateful vicissitudes. For the newer generation of Bulgarians, his revolutionary-sounding name means very little. For the beginning of the 20th century, when Bulgaria was reviving and the struggle for the unification of our lands in Thrace and Macedonia continued, the name Pushkarov was completely covered with his innovative and revolutionary spirit.

For a person who shouldered a rifle and dedicated himself to the national liberation struggle, to educational and awakening, and later to scientific activity, there can be no easy and straight paths. Pushkarov's life until the last time was thorny, he became a victim of his faith and had the sad honor of being recognized posthumously, although his entire activity was filled with constant struggle and incredible work in the name of the Fatherland, the disclosure of the riches of the Bulgarian land and its preservation and protection.

Nikola Pushkarov was born in the town of Pirdop in a family of prominent Bulgarian revolutionaries and supporters of freedom and national independence. Great historical figures and factors influenced his development - his father Petko Pushkarov, a prominent public figure before and after the Liberation, his teacher - the writer Todor Vlaikov, the legendary voivode Gotse Delchev and the famous German (Ramanov) Institute in Munich, where he conducted a long-term specialization, the progressive intellectual and scientific elite from Sofia University and BAS, with whom he did not break his ties from his studentship until the end of his life. The implacable spirit towards injustice caused him to publish newspapers and organize progressive student struggles even in high school, which is why he successively changed high schools in Sofia, Sevlievo, Plovdiv and Tarnovo. Although an excellent student, wherever he was transferred, his report card said that he was a "rebel against the authorities". He graduated in Varna, where he became friends with Metodiy Popov, (later academician and founder of the Institute of Biology) and Vasil Kolarov. In 1896, he was accepted as a natural science student at the Higher School in Sofia, and during his studies he became a favorite of Academician Georgi Bonchev, who invited him to study together the petrography of the Central Balkans, Vitosha and Rila. In the Higher

School (Sofia University) he was the founder, together with M. Popov, R. Popov and Iv. Ploshakov (later famous scientists) and leader of the Society of Naturalist Students, a participant in the student struggles against the Radoslav regime. As a graduate student, after graduation he received a personal invitation from the then Minister of Education Petko Karavelov to work in the Ministry, but he refused and returned to his hometown Pirdop as a teacher. He developed a wide cultural, educational and political activity, for which he was brought to court. Personally, Petko Karavelov, already as Prime Minister intervened and the charges were dropped. As a teacher and organizer of the teacher's movement in Pirdop, Plovdiv, Sofia, Pleven and Vidin and Chairman of the teacher's association in the Central Highlands, he was fired more than once. He was also persecuted as a prominent lecturer of the cultural and educational societies and founder of the "Oralo" Mutual Aid Society with 63 members in his hometown, because he protected them from moneylenders. Pushkarov organized his fellow teachers and in one month they made 30,000 bricks for the company's building for free. It should be noted that in Vidin he is co-editor of Zdrave magazine, founded the National University and is a beloved lecturer, an authority of the city. Here he carries out hydrological studies and develops a project for the city's water supply. Unfortunately, this project is carried out by the highly paid Austrian professor Farhamper, who receives the huge fee. Pushkarov recommends building suitable dykes and belts in the Vidin field to protect the land from the waters of the Danube and the north wind. Wherever he taught, he remained in the minds of his colleagues and students as a teacher with "great intelligence and education, high culture and knowledge". Despite the low pay of his teaching job, he helps his poor but studious students with the means to complete their education. In 1902, during a personal meeting with Gotse Delchev and on his advice, he decided to go to the southwestern Bulgarian lands (Macedonia), located within the borders of the Ottoman Empire. His desire is to work in Bitola as a teacher and participate in the fight against pro-Serbian propaganda and the "Serboman" question. By an act of the Bulgarian Exarchate, he was sent as a natural history teacher to the Men's Pedagogical School in Skopje to lead and participate in the preparation of the Ilinden-Preobrazhensky Uprising. Here he began to publish the magazine "Osvobozhdenie", together with Struga-born teacher at the Skopje Girls' School Slavka Chakarova, who four years later became his wife, as well as Lyuba Kuileva from Veles and Yanka Kanevcheva from Ohrid. The revolutionary environment in which Pushkarov worked included fighters, teachers, craftsmen, young people from the outskirts of Skopje and the nearby towns and villages - Tetovo, Kumanovo, Kočani, Kratovo and Palanka. The revolutionary and printing activity did not remain hidden from the Turkish administration, but thanks to the brave women around him and his companions in propaganda and struggle - the teacher Dimitar Galev from Skopje, Iliya Rashtinov from Veles, Kiril Stoyanov from Mavrovo and Dimitar Ganchev from Ruse, he remained unharmed after several searches and searches. At the same time, he was elected chairman of the Skopje Revolutionary Committee. He roamed as a voivode with a band of 11 men in the mountains and the surroundings of Skopje to avenge the "depredations of the Turks" and organize the uprising, and his love of petrography led him to collect rock samples in his backpack. For some time he returned to the Principality to attract more Chetniks and worked on sending weapons and "hell machines" and the production of cyanpotassium at Sofia University. During the uprising, the squad grew to 25 insurgents, armed it with the necessary weapons - 200 bombs, 7 loads of dynamite and hydrogen cyanide. Already on August 3, he organized the explosion of a train composition of 32 wagons, the Skopje-Veles railway line, destroyed the security department of the bridge over the Vardar river, fought battles with Turkish troops in the Veterski and Gyurishki monasteries and other areas. The just struggle for independence of Bulgarians and

Turks is briefly reflected in his memories: "We fought for the freedom of both". After the failure of the uprising, Voivode Pushkarov in the fall and winter of 1903-1904 organized several meetings of the Skopje Revolutionary District together with Hristo Matov and Dr. Hristo Tatarchev, how to continue the struggle. Returning to Bulgaria, he maintains a relationship with Yane Sandanski and hides him in his home in Sofia on "Ivac Voivoda" Street. As one of the leaders of the four and an active participant in VMORO, on March 9, 1919, Pushkarov was among the 17 former voivodes, including Gyorche Petrov, Mihail Gerdzhikov, and others, who signed a special appeal to the Macedonian population and emigration to Bulgaria. His relationship with these revolutionaries and his joint activity with them did not go unnoticed later in scientific circles by cabinet professors. The fear of the patriot and the fighter for a united Bulgaria and the envy of the innovative scientist and "uncomfortable opponent in science" makes them pursue him all their lives. His work as a teacher in his hometown and especially in the period 1904-1908 in the country and as a deputy head in the Ministry of Public Education in 1909-1911 could not stop him from thinking about the scientific ideas planted by his mentor and Professor at the University of Academician Georgi Bonchev. Without being appointed as a researcher, in 1909 he wrote the capital work "Formation of the soil" and thus started the study of the genesis and origin of soils. After this period, active scientific research, journalistic and public activity began. He edited the magazines *Natural Science and Geography*, *Chemistry and Industry*, where the biggest representatives of science at that time collaborated - As. Zlatarov, G. Bonchev, P. Bakalov, Z. Karaoglanov, M. Dimitrov, St. Petkov, R. Popov, Zh. Radev, G. Shishkov and other famous natural scientists. Pushkarov was the first editor of the "Journal of Agricultural Testing Institutes in Bulgaria", the first translator in our country of "Origin of Species" by C. Darwin. He was several times elected Chairman of the Bulgarian Nature Protection Society and is one of the founders of the Geological Society. At his suggestion to the Minister of Trade and Agriculture, in 1911 a Soil Science Section was opened at the Agricultural Experiment Station in Sofia. This historical date is considered the birth of Bulgarian soil science, and the head of the section is the first soil scientist in our country. Today, when so many years have passed, his statements about the origin and classification of soils, mineral fertilization and fertilizer requirements of crops, acidification, salinization, land reclamation and tillage, drought control, chemicalization and irrigation, zoning of agricultural crops are still not outdated. etc. All these topics are still relevant today. Pushkarov was the first scientist in our country who established the basic regularities of the geographical distribution of soils and proved the agro-ecological approach to their use. He developed the first National Soil Map and the first methodology for credit assessment of agricultural land, which he implemented in the municipalities. Thus, he is rightfully recognized by the new generations of researchers as the Patriarch of Bulgarian soil science.

After specializing in Germany in 1914-1915, he became thoroughly acquainted with the Russian Dokuchaev school, and continued to carry out a large-scale scientific program for the study of soil and land resources in the country. The recollections of one of them - the agronomist Georgi Ivanov Popov - bear witness to the studies and meetings with the farmers. He points to Pushkarov's "lively interest and direct involvement in the work" that he showed during soil studies and the implementation of the methodology for bonification of agricultural lands in settlements. Pushkarov consistently published soil-geological and soil sketches, works and studies for different regions of Bulgaria. Broad general culture and comprehensive agro-soil and agro-biological research established his great authority as a scientist and researcher at the Agricultural Experiment Station, and in 1919 he was appointed director of the station, after a unanimous vote of his colleagues. According to his report, the station was reformed into an Institute in 1920 with

8 scientific departments. Academician Kiril Pavlov highly appreciates his activity as director: "It was a real happiness for the Institute that during this period a person like N. Pushkarov stood at the head. Excellently prepared, persistent as a scientist, progressive and good, but firm and consistent person, managed to raise the Institute to a great height as a scientific research institution in a short time". The coup of June 9, 1923 found the Institute in a great upswing, for which Pushkarov has merits. According to Academician M. Dimitrov's recollections, shortly before the coup, he led a scientific controversy with two scientists, one of whom was Prof. Yanaki Mollov. After becoming the Minister of Agriculture, he dismissed Pushkarov for political reasons and took away the land he managed to secure for the construction of the institute's building. The prominent Bulgarian historian and philosopher also wrote: "The selfless and selfless scientist was deprived not only of the opportunity to continue his scientific research, but also of a piece of bread. Pushkarov was proud, he did not go to pray to anyone, nor did he send anyone to pray for his return. He spent dark days when his family had nothing to eat". As a result of the difficult living conditions, the illness and death of his only 20-year-old daughter. He was reinstated after 4 years, in 1926, as head of the Soil Department. With extreme material and personnel shortages, very often alone and with his own funds, he continues the soil surveys in the country for their comprehensive study. Promotes the ideas of collective cultivation and land consolidation. In 1931, he issued the first Soil Map of Bulgaria in M. 1:500000 in Bulgarian and German, for which he took out a loan from the Yuchbunar Popular Bank against a mortgage on his home, which he paid until the end of his life. This example of selflessness and devotion to science, the Bulgarian land and its people far exceeds ordinary moral norms. At that time, his works were read in Europe and the German professor H. Shtreme used Pushkarov's Soil Map to develop a Soil Map of Europe. He promoted the latest works of a number of Russian and Western scientists (Gedroits, Sigmond, Lemerman, Blank, etc.). Despite everything done by the great scientist, after the political coup on June 19, 1934, the opportunity for research work was again taken away by the reactionary representatives of the Faculty of Agronomy and Forestry, headed by Yanaki Mollov, who did not share his views on science. He retired prematurely, but continued to explore the country's soils with his own funds. In 1940, progressive specialists from the Ministry of Agriculture appointed Pushkarov as a laborer (what a humiliation!) to the Soil Department of the Agricultural Testing Institute with the task of developing a new soil map. Recently, the fascist minister Hr. Petrov, who immediately removed Pushkarov from work on the denunciation of one of his students/late professor in the Public Polytechnic of Hydrotechnical Faculty of today's UASG/ and a group around him. Deeply embittered by another blow, he continued to work in his home study and alone, with the meager pension and with the help of many good people in places, continued to explore the soils of the Danube plain. He spent his last savings, but the second national soil map in Bulgaria remains unfinished. After decades of soil research across the country, it continues to be respected and sought after by science and practice. At the invitation of residents of the village of Ladzhene (today a district of Velingrad), in 1942 he explored and discovered the warm mineral springs for the teachers' sanatorium that was built at the time. In the same year, he gave serious support to the commissioning of the chalk-lime production in the town of Slivnitsa. Although in very poor health, he worked until the end to issue the Second National Soil Map and support his family. He died at his desk on 19.02.1943 in deprivation and misery, but proud and with dignity, with an honestly traveled path in science and in the fight against internal and external enemies. Today, when we turn the pages of his many works and admire his deeds, we ask ourselves: why did his life go like this. The answer is one - Pushkarov was a person with innovative solutions to vitally important and national problems, a

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person who was a thorn in the side of pygmies in science and politics. And the brighter the personality, the more it irritates small and inferior people. This irritated them, and they held him in their sights, although he never belonged to any party, but was a democrat by nature, had purely populist ideas, and defended the enslaved, the poor, and the wronged with gun, pen, and word. This is never forgiven - before and now. This is what his fellow citizen, the poet Veselin Andreev, wrote: "Pushkarov was a strong man. Life had taught him to endure everything firmly with inner conviction and pride. He had suffered not only in his youth. They harassed him even in his advanced age, when with youthful energy, with admirably devoted love of his science—what a splendid example for the present generations! - traveled around the country in murderous conditions, did research". Pushkarov did not live to receive recognition for his major work. Only when the Bulgarian-Soviet soil expedition took place in 1947, prominent Soviet scientists and academicians I.P. Gerasimov and I.N. Antipov-Karataev gave a high and well-deserved assessment of his overall activity. In 1948, his name was given to one of the most important agricultural institutes in our country - the Institute of Soil Science. 104 years after the writing of the capital work "Soil formation", the UN General Assembly, with a Resolution of the 68th session of 20.12.2013, decided that December 5 will be celebrated annually as World Soil Day, and 2015 will be declared the International Year of the Soil. What international recognition and appreciation for the importance of this unique natural resource for feeding the population of the Planet! What foresight of the great Bulgarian scientist Nikola Pushkarov to start the study of soil and land resources more than a century ago!

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# BULGARIAN JOURNAL OF SOIL SCIENCE®

## INSTRUCTIONS FOR AUTHORS

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### Manuscript Submission

Submission of the manuscripts implies that they have not been published before nor are currently submitted or considered for publication in any other journals, which should be declared in **cover letter** by the authors.

*The manuscripts must be submitted in electronic version to the Editorial e-mail address: [Journal@bsss.bg](mailto:Journal@bsss.bg) and [bulsoil@gmail.com](mailto:bulsoil@gmail.com)*

### Language

Manuscripts should be written in clear and grammatically correct English. British or American English spelling and terminology must be used. If English is not your native language we strongly recommend that the text of your paper be checked by a native speaker or a professional science editor before submission.

### Types of Manuscripts

Bulgarian Journal of Soil Science publishes four categories of papers and the authors must indicate the category of their submission in the cover letter:

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### General presentation of the manuscripts

The manuscripts should be double-spaced (1800 characters per page), without section breaks, on A4 (210 x 297 mm) paper size, with margins of at least 2.5 cm at the top, bottom and sides. Lines should be numbered in the margins with a continuous numbering from the start of the manuscript. All pages should be numbered consecutively in the bottom, right-hand corner.

**The manuscript should be arranged as follows:**

### Title Page

The title page should include:

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- The affiliate institutions(s) and address(es) of the author(s);
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### **Abstract**

Please provide an abstract up to 1500 characters that includes a clear statement of the research objectives, a short overview of the methods used, the most important results and a conclusion. No references and abbreviations should be used in the Abstract.

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### **Order of sections**

The standard order of sections should be: Introduction, Material and Methods, Results, Discussion (or Results and Discussion), Conclusion, Acknowledgements, and References.

**The content of the manuscript should be structured in accordance with the following criteria**

**Introduction.** It should present a general framework of the topic in justifying why the authors have performed the study. State-of- art on the research topic has to be presented briefly to derive the novelty of the study and to show its relation to the preceding works. A distinct paragraph at the end of this chapter should state the objectives of the work.

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### **Conclusion.**

This chapter or subsection of the discussion chapter should be concise and derived from the results of the research and the discussion presented. General statements are not allowed.

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Please always use internationally accepted signs and symbols for units, SI units. Genus and species names should be in italics.

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  - Abbreviations should be defined at first mention and used consistently thereafter.
  - Footnotes can be used to give additional information. Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.
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### **Tables**

The tables should always be cited in text in consecutive numerical order and must be numbered using Arabic numerals. Each table must be as simple as possible, double-spaced, of size no bigger than 12 x 18 cm. For each table, please supply a table caption (title) describing its content. Footnotes to tables should be indicated by superscript lower-case letters or asterisks and included beneath the table body.

Example: **Table 1.** *Soil indexes in the territory (2015–2016).*

### **Illustrations**

All illustrations, including charts and photos, must be labelled as Figures. They must be numbered consecutively and should be provided with an explanatory legend on a separate sheet entitled ‘Figure captions’. They should conform to the size of the typed area (12 x 18 cm) which is the maximum size for the illustrations. Magnification should be shown by scale bars. Files in XLS, TIF, JPG and WMF formats are accepted. After the paper is accepted the editors will ask for figures in JPG or TIF format with 300 Dpi. Colour illustrations are accepted without fees for electronic publications. The editors reserve the right to decide what figures are justified and can be published in RGB colour (other figures will be removed or published in black and white).

Example: **Fig. 1.** *Soil map of the study area.*

### **Citation and References**

From July 2021, Bulgarian Journal of Soil Science adopts the APA (American Psychological Association) bibliographic style (7th edition – 2020). APA Referencing Basics: In-Text Citation.

#### **Citation**

Cite references in the text by name and year in parentheses as follows: Bauer (1932) or Bauer, Smith (1951) or (Bauer, 1930; Smith and Albert, 1931, 1935; Bauer et al., 1965) or Steffanic et al. (1951), up to two authors, give the names; for more than two authors, give the name of the first author followed by ‘et al.’. Check each reference with the original article and refer to it in the text. All authors of a paper should be cited in the list of references. References ‘in press’ shall only be cited when they have been accepted for publication.

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If the author of the cited source is unknown, the first few words of the reference should be used. This is usually the title of the source. If this is the title of a book, periodical, brochure or report, it should be italicised. For example: (IUSS- Working group, 2015). If this is the title of an article, chapter or web page, it should be in quotation marks. For example: (“APA Citation”, 2017). Citing a group or organization: (World Olympic Committee, 2018).

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For example: (Hristov, 2017a) Or (Hristov, 2017b). In case of papers written in other than Latin letters, if there is an English (or German, French) title in the summary, it may be used. If there is not such a summary, the author's name must be transcribed (using British English transcription) and the title of the paper must be translated into English. This should be noted in round brackets at the end of the paragraph, for instance: (In Bulgarian).

### **Reference list**

References should be arranged first alphabetically and then further sorted chronologically if necessary. Some examples for references to journal publications, books, chapters in edited books and electronic publications or databases:

#### **Journal articles**

- Atanassova, I., Doerr S. (2010). Organic compounds of different extractability in total solvent extracts from soils of contrasting water repellency. *European Journal of Soil Science*, 61(1), 298-313.
- Calderón, H. W. (1982). The content and composition of humus in arid alluvial soil of Peru. *Soil Science, Academy of Sciences of USSR*, Moscow, 8(1), 53-59. (in Russian).

#### **Books**

- Sparks, D. L. (2002). *Environmental Soil Chemistry*, 2nd Edition, Academic Press, San Diego, CA.
- Lidanski, T. (1998). *Statistical methods in biology and agriculture*. Zemizdat, Sofia (in Bulgarian).

#### **Book chapters or conference proceedings**

- Maison, S. P. (1993). Advanced soil remediation. In: *Plants that accumulate heavy metals* (P. D. Tox, ed.). CAB Int., Wallingwood, USA, 291-307.
- Kostov, D., Radev, H. Peeva, M. (2008). Study of newly introduced cultivars of green and red apples. In: *Sustainable Fruit Growing: From Plant to Product, Proceedings of International Scientific Conference, Bulgaria*, 46-53.
- Petrova, R. (1989). Loss of Humus Soil in the Process of Reclamation of Damaged Terrains. In: *Fourth National Conference on Soil Science 'Problems of Soil Science in Intensive Agriculture'* (N. Sherbanova, R. Nacheva, Eds.). BSSS, N. Poushkarov Soil Science and Yield Programming Institute, Sofia, 203-206, (In Bulgarian).

#### **Online publications**

Lecoq, H. & Desbiez, C. (2012). Viruses of cucurbit crops in the Mediterranean region: an ever-changing picture. *Adv. Virus Res.*, 84, 67-126. <http://doi.org/10.1016/B978-0-12-394314-9.00003-8>.

#### **Dissertations and Philosophy Doctor Theses**

Hristova, M., 2013. content and availability of microelements-metals in Technogenic soils. Ph.D. Thesis. Agricultural Academy, ISSAPP 'N. Poushkarov', Sofia, 140. (In Bulgarian) .